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INTERACTION OF POLYCYCLIC  
AROMATIC HYDROCARBONS WITH  
DISSOLVED AND SEDIMENT ASSOCIATED  
HUMIC MATERIALS (NMR,  
FLUORESCENCE)

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**INTERACTION OF POLYCYCLIC AROMATIC HYDROCARBONS WITH  
DISSOLVED AND SEDIMENT ASSOCIATED HUMIC MATERIALS**

*University of New Hampshire*

PH.D. 1986

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INTERACTION OF POLYCYCLIC AROMATIC  
HYDROCARBONS WITH DISSOLVED AND  
SEDIMENT ASSOCIATED HUMIC MATERIALS

BY

THOMAS D. GAUTHIER  
B.S., Merrimack College, 1982

DISSERTATION

Submitted to the University of New Hampshire  
in Partial Fulfillment of  
the Requirements for the Degree of

Doctor of Philosophy

in

Chemistry

December, 1986



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This dissertation is dedicated  
to my parents

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ABSTRACT

INTERACTION OF POLYCYCLIC AROMATIC  
HYDROCARBONS WITH DISSOLVED AND  
SEDIMENT ASSOCIATED HUMIC MATERIALS

by

Thomas D. Gauthier  
University of New Hampshire, December, 1986

A fluorescence quenching method was developed for determining association constants of polycyclic aromatic hydrocarbons (PAH)s with dissolved and sediment bound humic materials. The technique is based upon the observation that PAH fluorescence in aqueous solution is quenched upon association with humic material. Association constants are derived from the fractional decrease in fluorescence intensity as a function of added humic material using Stern-Volmer plots. No separation is required and since the technique involves a ratio measurement the exact concentration of pollutant is not required. Anthracene-humic acid association constants normalized to the fraction of organic carbon in the sorbent ( $K_{oc}$ ), determined by fluorescence quenching, correlated quite well with  $K_{oc}$  estimates determined by a reversed-phase procedure. The long term reproducibility was  $\pm 3.8\%$  relative standard deviation.

The fluorescence quenching technique was used to investigate the effects of pH, ionic strength, and humic

acid composition on pyrene-humic acid  $K_{OC}$  values. Carbon, hydrogen, and nitrogen analysis plus UV, infrared, and  $^{13}C$  solid state nuclear magnetic resonance (NMR) spectroscopy were used to investigate compositional and structural variations in humic materials. Three measures of the degree of C=C bond formation (UV absorptivity at 272 nm, atomic H/C ratio, and fraction of aromatic carbon determined by  $^{13}C$  NMR spectroscopy) indicated that pyrene is sorbed preferentially by humic acids possessing a high degree of aromaticity. Solution ionic strength affected  $K_{OC}$  estimates in a complex manner. It was hypothesized that humic acid conformational changes and PAH salting out effects accounted for this behavior. Variations in the concentration of stock humic acid solution used in the fluorescence quenching experiments also produced a change in  $K_{OC}$  values. Variations in pH from 5 to 8 did not produce significant effects.

Measured  $K_{OC}$  values were similar for anthracene binding to a purified sediment sample and humic acid extracted from that sediment; however, after partial extraction with dilute base, anthracene sorption to the sediment increased by a factor of two. The residual organic matter after extraction is believed to be enriched in humin and responsible for the increased sorption.

In a separate study, determination of PAH in Great Bay sediments by extraction and capillary gas chromatography produced a very good correlation between the total PAH

concentration and the organic matter content of the  
sediments.

## CHAPTER 1

### INTRODUCTION

The ultimate fate of pollutants released into the environment is highly dependent upon their sorptive behavior. Many of the chemicals that represent the greatest potential hazard to the environment are very water insoluble and, therefore, tend to associate with sediments, suspended solids, and dissolved organic matter (DOM). Sorption to suspended solids and DOM increases the apparent water solubility of contaminants and to a large extent controls distribution in the environment (Lee et al., 1982; Pavlou and Dexter, 1979). Strongly sorbed contaminants have the potential of accumulating to dangerous concentrations in areas of high sedimentation (Jaffe and Hites, 1986; Neff, 1979).

In addition to controlling transport, it has been suggested that sorption to solids may alter rates of removal or alteration of contaminants by volatilization, photolysis, and chemical or biological degradation. It is generally recognized that contaminants bound to sediments are less bioavailable and thus have a reduced potential for bioaccumulation (Varanasi, 1985). As a result, accurate assessment of the toxicological impact of a chemical contaminant requires information concerning its distribution between various sorbed and dissolved states.

The term sorption will be used here to describe any accumulation of dissolved pollutants by sediments, soils or DOM. The bulk of naturally occurring organic matter in sediments, soils, and natural waters is comprised of humic substances. Humic substances are amorphous, acidic polymers resulting from the condensation of plant and animal remains. They are extremely complex materials and are classified on the basis of solubility into fulvic acids (soluble in acid and base), humic acids (soluble in base only), and humin (insoluble in acid and base) (Schnitzer and Khan, 1972). The fact that DOM may exist as microparticulates, colloidal suspensions, or in truly dissolved form complicates the definition of DOM. Generally, DOM is defined operationally upon its method of isolation prior to determination.

The sorption process is assumed to be an equilibrium process which can be described graphically by a sorption isotherm, i.e., a plot of pollutant concentration in the sorbed phase versus the pollutant concentration in solution. Sorption isotherms are typically measured at a constant sorbent concentration and at a constant temperature. The several models developed to describe this relationship have been reviewed (Voice and Weber, 1983). A common characteristic among each of the models is that in the environmentally important region of low solution concentration a linear sorption isotherm is predicted. Linear sorption isotherms can be described by a single distribution coefficient,  $K_d$ , which is simply the slope

of the isotherm, usually expressed in units of mL/g.

For hydrophobic organic compounds, sorption depends primarily on the organic matter content of the sorbent (Lambert, 1968; Karickhoff et al., 1979; Schwarzenbach and Westall, 1981). Lambert observed that there was a relationship between the biological activity of certain herbicides and the organic matter content of soils. He suggested that the organic matter acted as an immiscible organic solvent into which the herbicides could partition from soil water (Lambert, 1968). A similar relationship has been observed by several researchers investigating the sorptive behavior of nonionic organic pollutants on suspended sediments and soils (Karickhoff et al., 1979; Means et al., 1980). When distribution coefficients were normalized to the fraction of organic carbon in the sorbent, they were shown to remain relatively constant for a particular compound despite variations in the sorbent materials. This has resulted in the convention of reporting the distribution coefficient,  $K_d$ , in the form of a  $K_{oc}$  value which is simply  $K_d$  divided by the fraction of organic carbon in the sorbent ( $f_{oc}$ ).

$$K_{oc} = K_d / f_{oc} \quad (1)$$

In addition to establishing a convenient convention for reporting distribution coefficients,  $K_{oc}$  values were found to strongly correlate with octanol-water partition

coefficients ( $K_{ow}$ ). Karickhoff (1981) provided a theoretical explanation for this relationship based upon the fugacity or "escaping tendency" of the solute from the aqueous and organic matter phases. Both Karickhoff and Lambert (1968) view the natural organic matter as an organic phase into which solutes may partition from the aqueous phase. For hydrophobic solutes, the magnitude of the  $K_{oc}$  value is dominated by the aqueous phase fugacity coefficient which is inversely related to the aqueous solubility of the solute (S). Analogously, the magnitude of the  $K_{ow}$  value is also dominated by the same aqueous phase fugacity coefficient and so  $K_{oc}$  and  $K_{ow}$  are related by this term. Briggs (1981) has reviewed this subject and suggested that any pair of organic solvent-water partition coefficients should be related by a general log-log relationship:

$$\log K_1 = a \log K_2 + b \quad (2)$$

Hence, if the bioconcentration factor (BCF) is viewed as a partitioning between water and lipid phases, then  $K_{oc}$ ,  $K_{ow}$ , S, and BCF should all be related by some form of the above equation.

A number of researchers have attempted to define these relationships and have done so with moderate to good success, although each has found different empirical constants (Karickhoff et al., 1979; Means et al., 1980;



Schwarzenbach and Westall, 1981; Briggs, 1981). In general,  $K_{ow}$  values appear to provide the best estimates of  $K_{oc}$  values. However, in view of the fact that the magnitude of the constants vary and log-log plots are inherently insensitive, such estimates should be used with caution. Reasons suggested for equation variability include differences in experimental techniques, difficulties in determining  $K_{ow}$  values, and variations in the chemical and physical nature of the humic material.

Several authors have investigated DOM from different sources. Landrum et al. (1984) found considerable variation in partition coefficients for several organic pollutants bound to DOM from different sources, although the amount of DOM in the various natural waters was not very different. Whitehouse (1985) observed that the extent of PAH/DOM interaction in the aqueous phase was not simply a function of DOM concentration but also a function of the quality of the DOM with the underlying chemical composition undoubtedly a primary factor. Carter and Suffett (1982) reported similar variations when they studied the binding of DDT to dissolved humic materials. Slopes of the association curves varied by as much as a factor of 4 depending upon the source of the dissolved humic material.

The quality, or chemical and structural characteristics of humic and fulvic acids, have been shown to vary considerably on a geographic basis. In the past, infrared spectroscopy (Stevenson and Goh, 1971), elemental and

functional group analysis, and conventional NMR have been useful in elucidating the structural features of humic materials (Hatcher et al., 1980a; Dereppe et al., 1980; Wilson, 1981). More recently, NMR has gained increased popularity with the advent of fourier transform (FT), cross polarization (CP), and magic angle spinning (MAS) techniques (Mikinis et al., 1979; Hatcher et al., 1980; 1981; Barron and Wilson, 1981). Although the extreme complexity and inhomogeneity of humic and fulvic acids precludes determining exact structures, some generalizations can be made concerning differences due to geographic origin. In general, marine derived humic material relative to terrestrial derived humic material is less aromatic, more aliphatic, less highly condensed, poorer in phenolic groups, poorer in carbon, richer in nitrogen, sulfur, hydrogen, carbonyl groups, and carboxylic acid groups (Jackson, 1975).

Despite these differences, little work has been done to characterize the dependence of sorption on organic matter structure and composition. In fact, for simplicity, many still prefer to regard the organic matter as a homogeneous phase. In recent years, Chiou et al. have championed this view and cite correlations of solute solubility and  $K_{oc}$ , linear sorption isotherms, the absence of competitive effects, and low heats of sorption as support for this model (Chiou et al., 1979; 1981; 1983; 1984; and 1986). However this view has been criticized on the basis that the evidence does not exclude a physical adsorption model (Kyle, 1980;

Mingelgrin and Gerstl, 1983; MacIntyre and Smith, 1984).

Compared to sediments and suspended solids, DOM is equally effective in binding contaminants and controlling their transport and fate in natural waters (Carter and Suffet, 1982; Wijayaratne and Means, 1984a; Landrum et al., 1984; McCarthy and Jimenez, 1985a; Chiou et al., 1986). In 0.5% sodium humate solution the solubility of DDT was found to be at least 20 times the solubility in distilled water (Wershaw et al., 1969). Similarly, cholesterol binds strongly to DOM and is stabilized in the aqueous phase (Hassett and Anderson, 1979). The herbicide atrazine binds to DOM and exhibits chromatographic elution behavior characteristic of the DOM rather than its normal elution behavior (Wijayaratne and Means, 1984). The volatilization of chloroform and trichloroethene was reduced in the presence of humic substances compared to purified water (Callaway et al., 1984). Humic substances have also been shown to participate in the photolysis of certain organics via transfer of electronic energy from triplet states (Zepp et al., 1985). The bioaccumulation of benzo [a] pyrene, a potent carcinogen, in bluegill sunfish was reduced 90% when bound to dissolved humic material (McCarthy and Jimenez, 1985b).

Although its relative concentration in aquatic systems is generally quite low, DOM can represent a large fraction of the total sorptive capacity of the system due to the large volume of solution. Using a revised CEPAC model,

Caron et al. (1985) showed that at a DOM concentration of 6.95 mg/L, the fraction of the hydrophobic pollutant DDT associated with DOM was greater than the fraction associated with suspended sediments or biota. This effect would be greatest in aquatic systems with high DOM concentrations and sandy sediments of low organic carbon contents.

Recently DOM has been implicated in the apparent anomolous effect of solids concentration on the laboratory determination of hydrophobic pollutant sorption to suspended sediments and soils (O'Connor and Connolly, 1980; Horzempa and DiToro, 1983; Voice et al., 1983; Booth and Grant, 1985). One possible explanation for the decrease in observed distribution coefficients as the solids-to-solution ratio increases can be based on changing concentrations of DOM (Voice and Weber, 1985; Gschwend and Wu, 1985). Often the distinction between sorbed and freely-dissolved states becomes ambiguous in the presence of DOM and many conventional methods of obtaining distribution coefficients are incapable of accurately separating freely-dissolved pollutant from pollutant bound to DOM.

Several methods are designed to physically separate freely-dissolved pollutant from pollutant bound to DOM. This separation step has been achieved via dialysis, ultrafiltration, gel permeation chromatography and reversed-phase chromatography. Each approach has its limitations. Equilibrium dialysis was used to evaluate the extent of binding between DDT and dissolved humic materials

(Carter and Suffet, 1982). A dialysis bag with a molecular weight cutoff of 1000 daltons retained the humic materials while allowing DDT to diffuse freely through the bag. Once equilibrium was reached, estimates of free and bound DDT were made by sampling inside and outside the bag. Dialysis has also been used to study the binding and dissociation of PAHs with dissolved humic materials (McCarthy and Jimenez, 1985a). The dialysis method, however, requires extremely long equilibration times, on the order of 4-7 days. In an attempt to use this method to study the binding of benzo[a]pyrene to dissolved humic acid, equilibration was not complete even after 11 days (Landrum et al., 1984). In addition bacterial growth can be a problem and sorbates frequently bind to the dialysis membrane.

Similarly, ultrafiltration has been employed to separate free from bound pollutant (Means and Wijayarathne, 1982; Wijayarathne and Means, 1984b). DOM from natural water samples were concentrated using a hollow fiber system having a nominal molecular weight cutoff of 5000 daltons. The enriched colloidal fraction was equilibrated with a solution spiked with a contaminant and then recirculated through the hollow fiber system. Both the colloidal and ultrafiltrate fractions were analyzed for the contaminant of interest to determine the fraction bound to DOM. The herbicides linuron and atrazine as well as several PAHs have been studied using this method. Other compounds were not amenable to this approach because of very high adsorption to the apparatus

(Hassett and Milicic, 1985).

Two different chromatographic methods have been utilized to separate free from DOM bound pollutant. The interaction of natural estuarine colloids with the herbicide atrazine was studied using gel permeation chromatographic techniques (Wijayarathne and Means, 1984a). Elution profiles of the <sup>14</sup>C labelled atrazine in the absence and presence of DOM revealed that atrazine is strongly bound by DOM. However the technique is subject to adsorption effects and no quantitative measurements were made.

Landrum et al. (1984) have described a reversed-phase separation using Sep-Pak C-18 cartridges to separate humic bound pollutant from freely-dissolved pollutant. In this method it is assumed that humic bound pollutant quantitatively passes through the cartridge while freely-dissolved material is sorbed. The concentration of freely-dissolved pollutant is estimated from measures of the initial concentration and the concentration of bound pollutant passing through the cartridge. Distribution coefficients determined using this method are, however, dependent upon flow rate, humic acid concentration, and equilibration times (Morehead et al., 1986).

In general, methods employing a separation step arouse concern that the separation may be incomplete and may underestimate the sorption capacity of the sorbent. Furthermore, the very act of separating free from bound pollutant may disturb the equilibrium and lead to variable

estimates of the distribution coefficient. In some cases, estimates of bound pollutant are derived from difference measurements involving initial and final concentrations of free pollutant. In view of the difficulties in separating complex natural organic substances, it seems inevitable that such difference measurements have large uncertainties.

A second approach to estimating the extent of interaction between the contaminant of interest and DOM involves measuring a parameter that is related directly to the aqueous chemical activity of the solute. Several investigators have measured the increase in apparent solubility of a pollutant in the presence of DOM. Chiou et al. (1986) added excess solute to an aqueous humic acid solution, centrifuged out the nondissolved solute, and extracted an aliquot of the supernatant to determine the apparent water solubility of the solute. These values were then used to estimate the fraction of pollutant sorbed assuming a partition like interaction. A more elegant approach utilized dynamic coupled column liquid chromatography to study the effect of DOM on the aqueous solubility of PAHs (Whitehouse, 1984). Haas and Kaplan (1985) used a closed loop recirculating gas isopiestic apparatus to determine the excess solubility of toluene in a humic acid solution compared to a distilled water solution.

Alternatively, measuring the decrease in volatilization of a compound from water in the presence of a sorbent can be used to derive distribution information. When the Henry's

law constant and the activity coefficient are known, the equilibrium gas concentration of a volatile organic compound in a closed system serves as a direct measure of its liquid concentration. The assumption made in this type of measurement is that the bound pollutant of interest is not volatile whereas the dissolved compound is. A number of variations on this approach have been described in the literature.

One approach is to sample the headspace above an equilibrated pollutant-DOM solution in a closed system and inject directly into the gas chromatograph. Headspace standards are used to determine the concentration of free species in solution and the bound pollutant is calculated by difference (Diachenko, 1981). A second approach assumes a linear sorption isotherm and involves adding equal amounts of contaminant to two distilled water solutions, one of which contains DOM. The vapor phase concentrations of contaminant in each system are then compared to derive the fraction bound. In this method, knowledge of the initial solute concentration is unnecessary. In an alternate procedure, nitrogen gas is bubbled through a pollutant/DOM solution in an open system and aliquots of the solution phase are analyzed before, during and after purging. With the appropriate mathematical interpretation, kinetic as well as equilibrium information can be obtained (Hassett and Milicic, 1985). The volatilization approach is limited to volatile contaminants and requires knowledge of individual



Henry's law constants for each contaminant.

Numerous researchers have reported that polycyclic aromatic hydrocarbons (PAH)s sorb strongly to both suspended sediments and DOM (Herbes, 1976; Means et al., 1980; Gjessing and Berglind, 1981; Wijayaratne and Means, 1984; McCarthy and Jimenez, 1985; Whitehouse, 1985; Morehead et al., 1986). PAHs are composed of two or more fused benzene rings lying in a single plane and range in molecular weight from 130 to 300 g/mole. As a class of compounds they are of interest because of their extreme toxicity and ubiquitous nature. Several PAHs such as benzo [a] pyrene and dibenz [a,h] anthracene are highly potent carcinogens (Harvey, 1982). PAHs are formed primarily through the pyrolysis of organic materials and are widely dispersed in the environment. They may be formed via direct biosynthesis by microbes and plants and they may be found in crude and refined oils. The majority of PAHs entering the aquatic environment originate from terrestrial sources and thus they tend to be localized in rivers, estuaries, and coastal marine environments where DOM may play a significant role in their transport and fate (Neff, 1979).

Described here is a new fluorescence quenching method to measure PAH binding to DOM. The method is based upon the observation that PAHs fluoresce in aqueous solution but not when associated with DOM as represented by dissolved humic and fulvic acids. As a consequence, the fraction of PAH associated with DOM may be determined directly from the

fractional decrease in fluorescence upon addition of humic substance. For compounds that fluoresce, this method offers advantages over previous methods which depend upon the separation of free from bound pollutant. In the fluorescence quenching experiment there is no separation step involved and the initial concentration of pollutant need not be determined. The method was first described by Shane et al. (1984). Since then others have used fluorescence quenching to follow the rate of association between PAHs and dissolved humic material (McCarthy and Jimenez, 1985). In addition, fluorescence polarization has been used to study the association of perylene with fulvic acid in glycerol/water mixtures but this technique is only applicable in systems where the fluorescence is not quenched by the presence of humic materials (Roemelt and Seitz, 1982).

The work described in this dissertation was conducted with two goals in mind. The first goal was to demonstrate the utility of the fluorescence quenching technique as a means of quantitating the degree of association between PAHs and DOM. Later this goal was extended to include associations between PAHs and sediments as well. The second major goal of this work was to employ the fluorescence quenching technique in the investigation of controlling parameters in PAH-DOM interactions. Variations in the magnitude of  $K_{oc}$  values were related to variations in pH, ionic strength, and structure and composition of humic

materials under controlled conditions.

In a separate study, trace level determinations of PAHs in the sediments of the Great Bay estuary in Portsmouth, NH were made in an attempt to correlate field residue data with variations in experimentally determined association constants.

## CHAPTER 2

### EXPERIMENTAL

#### Reagents.

The PAHs used in this study were pyrene (Aldrich, red label, 99+% pure), phenanthrene (Aldrich, red label, 98+% pure), and anthracene (Aldrich, gold label, 99.9+% pure). All were used without further purification. PAH solutions at concentrations below saturation were prepared by dissolving weighed amounts in water with the help of ultrasonic agitation (usually 24-48 hours). Radiolabelled [9,(10)-<sup>14</sup>C] anthracene (15.1 mCi/mole) from Amersham was purified by preparative thin layer chromatography before use. A stock solution was prepared by dissolving the purified anthracene in 1 mL of methylene chloride. Aqueous solutions were prepared just prior to use by adding 2 uL of stock anthracene to 250 mL of aqueous buffer. The anthracene stock solution was stored at 0°C in the dark. All other PAH solutions were stored in glass volumetric flasks in the dark. Adsorption of PAH to the walls of containers made it impractical to know the exact concentration in solution; however, a known PAH concentration was not required by the fluorescence quenching method.

Stock 0.1M buffer solutions were prepared using either sodium acetate (Mallinckrodt, anhydrous powder) and acetic

acid (VWR reagent grade) or monobasic potassium phosphate (Fisher) and dibasic sodium phosphate (Fisher) in proportions necessary to achieve the desired pH. These stock solutions were diluted to 0.01 M when used to buffer PAH solutions. Less concentrated buffer solutions were used in the investigation of ionic strength effects. Sodium chloride (Baker Analyzed, reagent grade) and calcium chloride (Fisher) were used to adjust ionic strength. Distilled water passed through an ultrapure mixed bed ion exchange resin (Barnstead D0809) was used as a source of purified water.

#### **Materials.**

Thirteen different humic acids and three different fulvic acids were examined in this study. The samples ranged in origin from a primarily marine environment to one that was terrestrial in nature. Eight humic materials were extracted from sediments sampled at different sites in and around the Great Bay estuary and off shore (Figure 1), four humic materials were extracted from soils, and one humic acid was obtained commercially from Aldrich Chemical Co. The three fulvic acids used had been extracted previously by others. Each of the materials are described briefly below.

With the exception of the humic material obtained commercially from Aldrich, which is designated ALD, each of the humic materials was extracted using the procedure

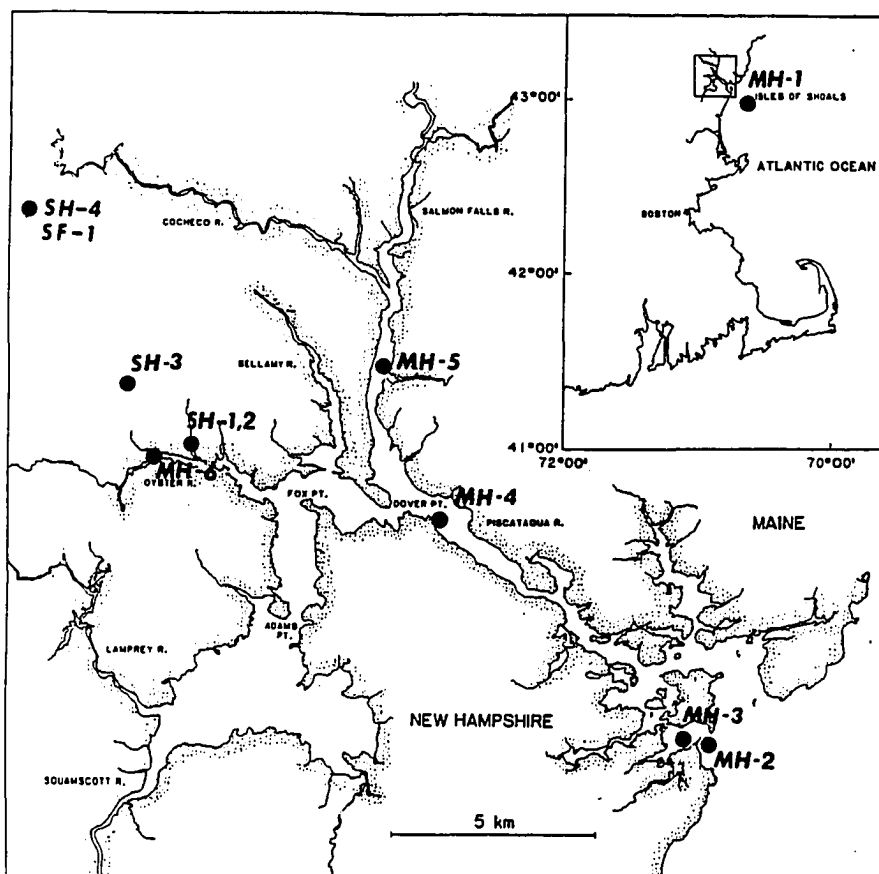


Figure 1. Map of Great Bay Estuary Depicting the Origin of Humic Acids Used in this Study.

described by Baur (1981). Briefly, the humic acids were extracted with 0.1 N NaOH for 2 hours in a nitrogen atmosphere, separated from fulvic acids by precipitation with HCl at a pH < 2.0, and purified with 5 successive 30 minute extractions with a 2.0% HF / 0.5% HCl solution. The purified product was then freeze-dried and stored in a desiccator until used. The isolation procedure for the Aldrich material is unknown.

The humic acid designated MH-1 was isolated from a sediment sampled 12 miles out of Portsmouth Harbor, NH near the Isles of Shoals in 103 ft of water. This sample was representative of a primarily marine-derived humic acid.

Humic acids MH-2 and MH-3 were isolated from sediments taken from Portsmouth Harbor, NH. These samples were primarily marine in nature although they are clearly subject to some terrestrial input.

Humic acids MH-4, MH-5, and MH-6 were extracted from sediments sampled at three different sites within the Great Bay estuary. These samples were primarily marine in nature but may be subject to some terrestrial input.

The humic acids designated SH-1 and SH-2 were isolated from sediments taken from the banks of the Oyster River in the same general vicinity but on two different dates; August 4, 1983 and October 1, 1985. These samples should be of primarily terrestrial origin but may be subject to some marine input.

Samples SH-3 and SH-4 were isolated from soils sampled

a few miles inland from Great Bay in Durham and Lee, NH, respectively. These materials represent terrestrially derived humic acids. The SH-4 humic acid has been previously characterized (Baur, 1981).

The humic acids designated SH-5 and SH-6 were extracted from a very dark lignite soil chosen because of its very high organic content. It is believed that the humic acids extracted from this soil have aged considerably longer than the previously described soil humic acids.

The fulvic acids were obtained from three different sources. One fulvic acid, designated SF-1, was isolated from a soil originating in Lee, NH, by acid extraction and repeated passage over Amberlite IR 120+ cation exchange resin (Baur, 1981). The second fulvic acid, designated SF-2, was isolated from a soil in N. Conway, NH. Its characteristics have been described (Weber and Wilson, 1975; Wilson and Weber, 1977). The third fulvic acid designated SF-3, was extracted from the Suwannee River in southeastern Georgia and has been comprehensively characterized (Thurman and Malcolm, 1983).

All humic materials were characterized by carbon, hydrogen, and nitrogen analysis and infrared and UV absorbance. Elemental analyses for each of the humic materials are found in Table 1. In addition, four of the humic acids (MH-5, SH-3, SH-4, and SH-5) were examined using  $^{13}\text{C}$  solid state NMR. They were chosen because they represent the greatest variations in terms of PAH



TABLE 1. Elemental Analyses (%), Atomic H/C and N/C Ratios, Absorptivities, and Pyrene  $K_{oc}$  Values of Humic Materials.

Humic Material	%C	%H	%N	H/C	C/N	ABS <sup>a</sup>	$K_{oc}$ <sup>b</sup>
MARINE HUMIC ACIDS							
MH-1	39.07	4.99	6.50	1.53	7.0	2.6	3.5
MH-2	47.22	6.13	7.64	1.56	7.2	2.7	5.0
MH-3	47.76	6.32	8.36	1.59	6.7	4.0	2.9
MH-4	45.10	5.65	6.69	1.50	7.9	2.9	6.4
MH-5	40.60	4.55	5.13	1.34	9.2	10.1	4.0
MH-6	48.58	5.47	5.77	1.35	9.8	6.4	5.5
SOIL HUMIC ACIDS							
SH-1	37.61	4.15	3.61	1.32	12.2	12.0	12.5
SH-2	38.99	4.28	3.96	1.32	11.5	13.0	8.0
SH-3	47.23	4.69	4.40	1.19	12.5	17.5	8.8
SH-4	54.15	4.67	3.51	1.03	18.0	23.0	16.1
SH-5	57.53	3.03	1.37	0.63	49.0	37.0	24.0
SH-6	54.66	2.98	1.28	0.65	50.0	46.0	32.0
SOIL FULVIC ACIDS							
SF-1	41.52	3.01	0.65	0.87	74.5	16.0	6.6
SF-2	53.1	3.24	0.90	0.73	69.0	10.0	5.4
SF-3	51.3	4.32	0.56	1.01	107.0	15.0	10.4
ALDRICH HUMIC ACID							
ALD	38.23	3.21	0.42	1.00	107.0	13.7	10.4

<sup>a</sup> Absorptivity  $\times 10^3$  (L/mg cm) at 272 nm

<sup>b</sup>  $K_{oc} \times 10^{-4}$  mL/g

binding capacity, UV and infrared absorbance, and elemental analysis and also because the isolated material was available in large enough amounts to obtain the NMR spectra.

Concentrated fulvic acid solutions were prepared by dissolving weighed amounts in water with the aid of ultrasonic agitation. Dissolution of humic acid required temporary elevation of pH. A working pH was then obtained by acidifying with HCl when necessary. Stock humic and fulvic acids were stored at 4 °C to retard bacterial growth.

The sediment sample used in this study was obtained from the banks of the Oyster River at low tide (OR sediment). This material is the same material from which the SH-2 humic acid was extracted. Only the surface layer (0-2 cm) of oxidized sediment was collected. Clean up of the sediment involved wet sieving: first through a coarse sieve to remove debris and then through a No. 230 USA Standard Testing Sieve (63 um opening). The sediment was then washed with distilled deionized water several times to remove soluble salts. The resulting material was air dried, homogenized with a mortar and pestle, and resieved. Sediment suspensions used in fluorescence quenching titrations were prepared by adding known amounts of sediment to 100 mL of distilled deionized water.

#### **Instrumentation.**

Solid state  $^{13}\text{C}$  NMR spectra of four humic samples were recorded at the Worcester Consortium NMR facility at Clark University. Spectra were run on a Bruker WM250 operating at a 62.9 MHz  $^{13}\text{C}$  observation frequency using magic angle spinning, a Doty probe and IBM solids accessory. Probe spinning speeds varied from 3.5-4.0 kHz. Pulse widths were 5.5 usec. Each spectrum is the result of 3000-13000 scans. No internal standard was used in order to maintain the integrity of the samples and, as a result, chemical shift assignments should be taken as  $\pm 10$  ppm. Each of the spectra revealed a strong relatively narrow absorbance at approximately 36 ppm due to aliphatic carbons. In determining chemical shifts this peak was assigned a chemical shift of 36 ppm and subsequent peaks were assigned relative to this peak.

Infrared spectra of the humic materials were recorded on a Perkin Elmer model 283 B infrared spectrophotometer operating in the % Transmission mode. Pellets were prepared by mixing 1.0 mg of freeze dried humic acid with 200 mg of KBr followed by drying in the oven for 2 hours at 100 °C prior to pelletizing and recording the spectra.

Elemental analyses were performed at the University Instrumentation Center on a Perkin Elmer model 240 B elemental analyzer. Absorbance measurements were made on a Bausch and Lomb Spectronic 200 recording spectrophotometer equipped with a deuterium lamp source.

Fluorescence measurements were made initially on a

Perkin Elmer MPF-44E spectrofluorometer on loan from the U.S. Geological Survey. Fluorescence intensity was measured as a function of added humic material at fixed wavelength with slits adjusted to give band widths of 2 nm on both excitation and emission monochromators. The excitation / emission wavelengths were 272/371 nm for pyrene; 250/380 nm for anthracene; and 288/364 nm for phenanthrene. Later, due to the return of the MPF-44E, fluorescence measurements were made on a Perkin Elmer Model 204 fluorescence spectrophotometer interfaced to an Apple IIE micro computer in order to increase the signal-to-noise ratio. This combination was capable of recording fluorescence intensities as an average of 225 data points.

#### Procedures.

**Fluorescence Quenching.** A typical fluorescence quenching experiment involved pre-equilibrating 2.25 mL of aqueous PAH solution, 0.250 mL of the appropriate aqueous buffer solution, and a micro stir bar in a 1x1x4 cm quartz fluorescence cuvet for a period of 30 minutes. By monitoring the initial decrease in fluorescence intensity as a function of time for a period of one hour, it was determined that 30 minutes was an adequate equilibration period so that further adsorption to the walls of the cuvet was negligible. An initial fluorescence intensity value was then recorded. Following this, a 10 or 50 uL aliquot of a 100 or 500 ppm stock humic acid solution was added to the

cuvet. Equilibration of the PAH was rapid, requiring less than one minute. However, the solution was stirred for 3-4 minutes and then allowed to stand quiescent for a minute before a second fluorescence intensity value was recorded. This step was repeated until a total of 5-6 aliquots of humic material had been added to the cuvet bringing the total concentration of humic material to 10-12 ppm. At this concentration, the fluorescence intensity of the initial PAH solution had decreased by approximately 50% implying that approximately 50% of the PAH was associated with humic material. The fulvic acids had weaker association constants and thus required a final concentration of 28-30 ppm humic material for an equivalent decrease in fluorescent intensity.

While adding humic material to solutions of PAH and during equilibration, the shutter of the spectrofluorometer was closed to avoid PAH photodegradation. The shutter was only opened for the actual intensity measurements. Since each intensity measurement required one minute, the total time of exposure to UV radiation was 5 - 6 minutes.

Absorbance measurements required to correct for inner filter effects were taken after each aliquot of humic material had been added during an actual experiment or at a later date on a solution of identical composition. These measurements were also used to calculate absorptivities. The slope of a plot of absorbance versus concentration of added humic material yields the absorptivity of the humic

material.

In some samples the measured fluorescence included a background component from the humic material. To correct for this the fluorescence of a solution containing humic substance only was measured using identical concentrations and instrumental conditions as in a PAH titration. The humic substance fluorescence intensity was subtracted from the total fluorescence intensity for PAH in the presence of humic substance prior to data reduction. The maximum humic material background fluorescence was only 3% of the total (Figure 2). However, to maximize PAH fluorescence relative to background, PAH concentrations at or near the solubility limit were used in this method. The background may be more troublesome when applying the fluorescence quenching method to PAHs of lower water solubilities than anthracene, pyrene, and phenanthrene. However, PAHs with lower water solubilities tend to bind more strongly to humic substances so that the humic concentrations required for the binding constant measurement are lower.

Two methods were used to investigate the effect of ionic strength on the association of pyrene with dissolved humic material. Each involved slight procedural changes in the fluorescence quenching technique. In one procedure, only the initial pyrene solution was adjusted to the appropriate ionic strength with sodium chloride; no adjustments were made to the stock humic acid solution. A second procedure involved adjusting both the initial aqueous

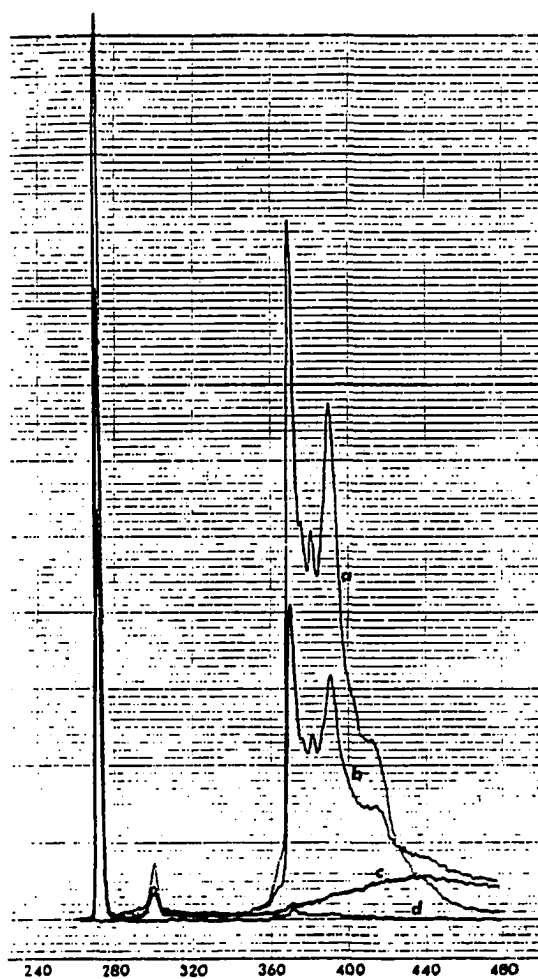


Figure 2. Fluorescence Spectra of a) Pyrene in the Absence of 28 ppm Fulvic Acid; b) Pyrene in the Presence of 28 ppm Fulvic Acid; c) a Solution of 28 ppm Fulvic Acid; and d) a Blank Solution.

pyrene solution and the stock humic acid solution with sodium chloride. The stock humic acid solution was then allowed to 'age' 24 hours prior to use.

For experiments involving sediments, a centrifugation step was added to the fluorescence quenching procedure in order to reduce effects due to scattered radiation. For example, after preequilibrating the aqueous PAH solution, a 50 uL aliquot of a sediment suspension was added to the cuvet. The contents of the cuvet were stirred for a period of five minutes and then centrifuged (Model CL International Clinical Centrifuge) for two minutes at 8800 rpm. A specially fabricated adapter allowed the cuvet to be placed directly in the centrifuge. After centrifugation, the fluorescence and absorbance of the solution were recorded and the titration was continued.

**Reversed-Phase.** The method used for comparison with PAH-DOM equilibrium association constants obtained here was based on a separation with C-18 Sep-Pak cartridges (Landrum et al., 1984). Briefly, the method consists of adding buffered aqueous radiolabelled anthracene solution and the appropriate amount of dissolved humic material to a Teflon bottle and equilibrating on a wrist action shaker for a period of 24 hours. After equilibration, free and bound anthracene were separated by passing an aliquot of the solution through a C-18 Sep-Pak cartridge (Waters Associates). It was assumed that anthracene bound to the humic material passed through the cartridge while free

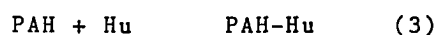


anthracene was retained. The concentrations of total anthracene and anthracene bound to DOM were determined in triplicate by mixing 2 mL aliquots of the solution with 10 mL of scintillation cocktail (Scinti Verse E, Fisher Scientific) in a vial and measuring the radioactivity on a Beckman LS-7000 liquid scintillation counter equipped with Automatic Quench Compensation. Background corrections were made on each of the samples. Estimates of free anthracene were obtained by difference.

Similarly, a batch equilibration procedure involving radiolabelled anthracene was used to evaluate the application of the fluorescence quenching technique to sediments. To a 50 mL oak ridge type Teflon centrifuge tube equipped with a screw cap was added 30 mL of buffered  $^{14}\text{C}$  labelled anthracene solution and the appropriate amount of sediment. The contents of the tube were shaken on a reciprocating shaker for a period of 24 hours. Three 2 mL aliquots of the suspension were then removed to determine the total concentration of anthracene in solution. The tubes were then resealed and centrifuged for a period of 45 minutes at 8800 rpm. After centrifugation, three 2 mL aliquots of the supernatant were transferred from the solution and used to determine the total concentration of anthracene in the supernatant. An additional three aliquots were passed through a C-18 Sep-Pak cartridge to determine the fraction of anthracene bound to DOM.

### Data Treatment.

The fluorescence quenching technique is based upon the observation that the intensity of fluorescence is proportionately decreased upon the addition of humic or fulvic acids. The association of PAH with humic or fulvic acids may be represented by the following equations:



$$K_b = \frac{[\text{PAH-Hu}]}{[\text{PAH}] [\text{Hu}]} \quad (4)$$

where PAH = polyaromatic hydrocarbon, Hu = humic substance, PAH-Hu = humic associated PAH, and  $K_b$  = equilibrium constant. The mass balance on the PAH is described in equation (5):

$$C_{\text{pah}} = [\text{PAH}] + [\text{PAH-Hu}] \quad (5)$$

where  $C_{\text{pah}}$  is the formal or total concentration of PAH. Combining equations (4) and (5) and rearranging yields equation (6).

$$C_{\text{pah}}/[\text{PAH}] = 1 + K_b[\text{Hu}] \quad (6)$$

If we assume that the fluorescence intensity is proportional to the concentration of free PAH in solution ( $[\text{PAH}]$ ), then

$$F_0/F = 1 + K_b[\text{Hu}] \quad (7)$$

where  $F_0$  is the fluorescence intensity in the absence of humic material and  $F$  is the fluorescence intensity in the presence of humic material. Since, at the concentrations used, a significant excess of humic acid was present,  $[\text{Hu}]$  was taken as the amount of added humic without correcting for the fraction of humic which was associated with PAH. Equation (7) is in the form of the Stern-Volmer equation (Lakowicz, 1983). The equilibrium binding constant can be obtained from the slope of a plot of  $F_0/F$  versus  $[\text{Hu}]$ .  $K_{oc}$  values were calculated by dividing  $K_b$  by the fraction of organic carbon in the humic material.

In general, there are three different types of quenching processes that can occur: static, dynamic, and apparent. In static quenching, a nonfluorescent complex is formed between the fluorophor and quencher. Equations (3) thru (7) represent a simple derivation of the Stern-Volmer equation resulting from static quenching. An alternative quenching process, also adequately described by the Stern-Volmer equation, is dynamic or collisional quenching. In dynamic quenching, the quencher must diffuse to the fluorophor during the lifetime of its excited state and nonradiatively deactivate that state. For dynamic quenching, the Stern-Volmer quenching constant is equal to the product of the bimolecular quenching rate constant and the fluorescence lifetime of the fluorophor in the absence

of quencher. This imposes constraints on the size of the Stern-Volmer constant as the bimolecular quenching rate constant has a diffusion controlled limit (Parker, 1968; Lakowicz, 1983).

Apparent quenching is not a quenching process at all but is rather due to an attenuation of the excitation beam and/or absorption of emitted radiation by an excess concentration of fluorophor or by the presence of an additional absorbing species in solution. This phenomenon is more commonly known as the "inner filter effect". Corrections can be made by taking into consideration the cell geometry and absorption characteristics of the solution. The higher concentrations of humic and fulvic acids used in the fluorescence quenching titrations absorbed light to a significant extent at both excitation and emission wavelengths. It was therefore necessary to correct for this effect based on the cell geometry shown in Figure 3 and the absorption characteristics of the solution as described by Parker (1968) in equation (8).

$$\frac{F_{\text{corr}}}{F_{\text{obs}}} = \frac{2.3 d A_{\text{ex}}}{1-10^{-dA_{\text{ex}}}} 10^{gA_{\text{em}}} \frac{2.3 s A_{\text{em}}}{1-10^{-sA_{\text{em}}}} \quad (8)$$

Where  $F_{\text{obs}}$  is the observed intensity,  $F_{\text{corr}}$  is the corrected intensity and  $A_{\text{ex}}$  and  $A_{\text{em}}$  are the

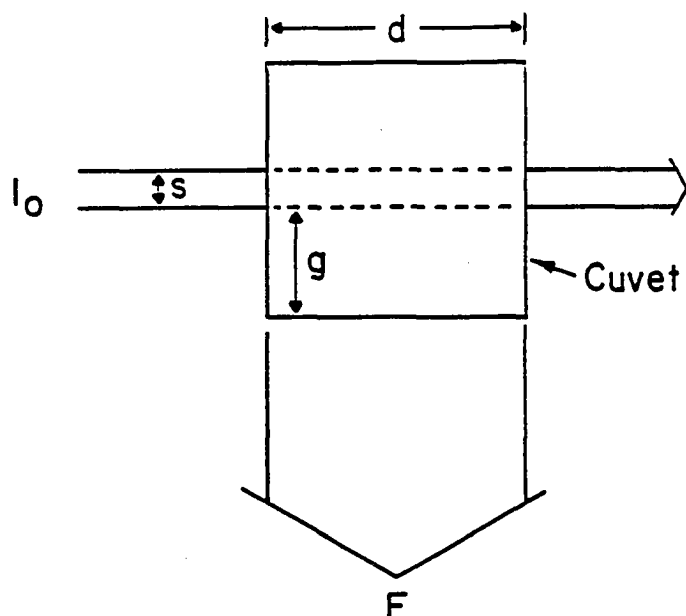


Figure 3. Assumed Geometry of Fluorescence Measurement with Parameters Used to Correct for the Inner Filter Effect.  $I_0$  Represents the Excitation Beam with Thickness,  $s$ , = 0.10 cm. The Distance from the Edge of the Sample Beam to the Edge of the Cuvet,  $g$ , = 0.40 cm. The  $F$  Represents the Observed Fluorescence Beam with Width = 1.00 cm.

absorbances per cm at the excitation and emission wavelengths respectively. The remaining terms,  $d$ ,  $g$ , and  $s$  depend upon the geometry of the measurement and are defined in Figure 3. The maximum value of the correction factor did not exceed 1.8 which is well within the recommended acceptable range.

## CHAPTER 3

### RESULTS AND DISCUSSION

#### Fluorescence Quenching Method for $K_{oc}$ Determinations

Three PAHs (pyrene, anthracene, and phenanthrene) were used to demonstrate the utility of the fluorescence quenching method for determining  $K_{oc}$  values. Some of their physical properties are listed in Table 2. In addition, pyrene was used to test the effects of pH and ionic strength on the magnitude of measured  $K_{oc}$  values. Anthracene and  $^{14}C$  labelled anthracene were used to compare  $K_{oc}$  values determined by fluorescence quenching and a reversed-phase procedure.

Initially, two fulvic acids and one humic acid were investigated. Each of these materials had been previously characterized and was available in quantity. Consequently, they provided an excellent source of materials with which to investigate the method. Later, it became apparent that structural and compositional variations in the humic material played a major role in determining the magnitude of PAH association constants. For this reason, a large variety of sediments and soils were sampled from the Great Bay area in southeastern NH in order to obtain humic materials from a variety of origins, i.e., primarily terrestrial or marine. Altogether, a total of 16 different humic and fulvic acids

Table 2. Physical Properties of Pyrene, Anthracene, and Phenanthrene.

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	Pyrene	Anthracene	Phenanthrene
Molecular Weight <sup>a</sup>	202.26	178.24	178.24
Melting Point ( C) <sup>a</sup>	156	216.3	101
Boiling Point ( C) <sup>a</sup>	393	340	340
Density (g/mL) <sup>a</sup>	1.271	1.283	0.980
Solubility (ug/L) <sup>b</sup>	135	73	1290
Kow (x10 <sup>-4</sup> ) <sup>c</sup>	8.4	2.6	2.3

<sup>a</sup>CRC Handbook of Chemistry and Physics (59<sup>th</sup> ed.)  
<sup>b</sup>J. Chem. Eng. Data 1977, 22, 399-402.  
<sup>c</sup>Chem. Rev. 1971, 71, 525-616.

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were studied including one fulvic acid that has been extensively characterized and one humic acid obtained commercially from Aldrich Chemical Co.

The results of a typical titration procedure are found in Table 3. Included are the concentrations of SF-1 fulvic acid titrant, absorbance readings, and fluorescence intensities after each addition to phenanthrene. The inner filter effect correction factor at each fulvic acid concentration is also calculated. Fluorescence intensities corrected for dilution effects and the inner filter effect are listed under the heading  $F_{\text{corr}}$ . As depicted in Figure 4, the resulting Stern-Volmer plot of  $F_0/F$  vs.  $[FA]$  is linear with a slope of  $3.2 \times 10^4$  mL/g.

Since both static and dynamic quenching can be described by linear Stern-Volmer plots, the data was analysed to determine which type of quenching was in operation. The measurement of fluorescence lifetimes in the presence and absence of quencher is the most definitive method to distinguish static and dynamic quenching. However, as we were unable to obtain lifetime measurements, we chose to evaluate the data for both conditions. Analysis of the data in Table 3 for the interaction of phenanthrene with SF-1 fulvic acid, resulted in a Stern-Volmer quenching constant ( $K_{\text{sv}}$ ) of  $3.2 \times 10^4$  mL/g. Assuming a molecular weight of 500 g/mol for the fulvic acid,  $K_{\text{sv}}$  becomes  $1.6 \times 10^4$   $M^{-1}$ . The fluorescence lifetime for phenanthrene in the absence of quencher is  $5.0 \times 10^{-8}$

Table 3. Fluorescence Quenching Titration Data for the Interaction of Phenanthrene with SF-1 Fulvic Acid.

[F.A.] (ppm)	A <sub>ex</sub>	A <sub>em</sub>	C.F.	F <sub>obs.</sub>	F <sub>corr.</sub>	F <sub>o</sub> /F
0.0	.065	.045	1.12	916	1031	1.00
3.9	.115	.065	1.21	743	910	1.13
7.8	.165	.085	1.31	614	817	1.26
11.6	.215	.105	1.41	518	747	1.38
15.4	.265	.120	1.51	439	685	1.50
19.1	.315	.140	1.62	379	639	1.61
22.7	.365	.155	1.73	327	594	1.73

A<sub>ex</sub> and A<sub>em</sub> are absorbance values recorded at the exciting and emitting wavelengths.  
 C.F. is the inner filter effect correction factor.  
 F<sub>obs.</sub> is the observed fluorescence intensity.  
 F<sub>corr.</sub> is the fluorescence intensity corrected for inner filter and dilution effects.

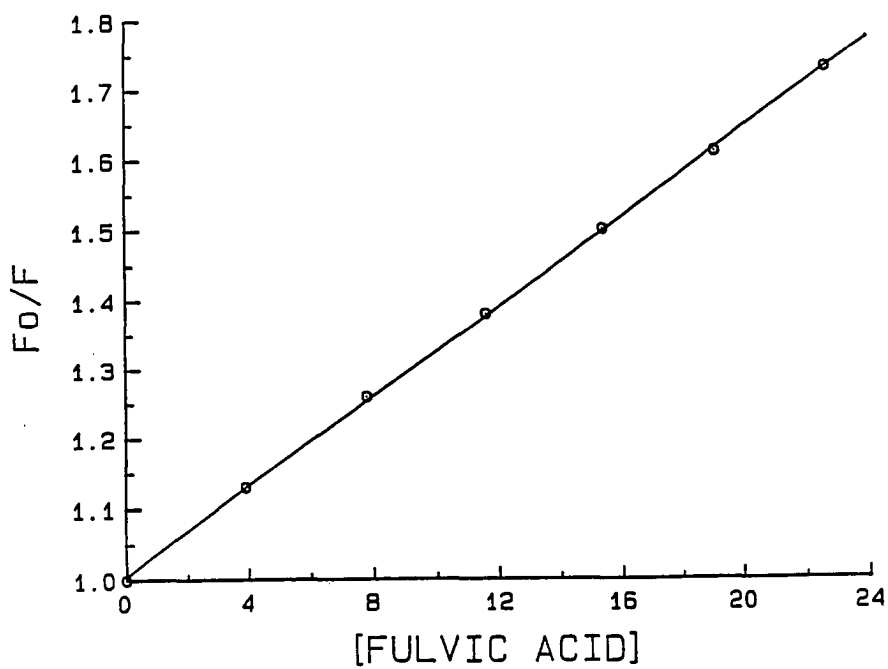


Figure 4. Stern-Volmer Plot for the Interaction of Phenanthrene with SF-1 Fulvic Acid.

seconds (Shizuka et al., 1978). Since, for a dynamic quenching process,  $K_{sv}$  is equal to the bimolecular quenching rate constant,  $K_q$ , times the fluorescence lifetime in the absence of quencher, the bimolecular quenching rate constant can be calculated. In this case  $K_q = 3.2 \times 10^{11} \text{ M}^{-1} \text{ sec}^{-1}$  which is unreasonably large for a diffusion controlled process in aqueous solution. A bimolecular quenching constant near  $1 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$  may be considered as the largest possible value in aqueous solution (Lakowicz, 1983). Analysis of other data sets leads to similar conclusions indicating that at most only a tiny fraction of the observed quenching can be dynamic.

If the Stern-Volmer quenching constants are interpreted as ground state association constants for PAH binding to humic material, then  $K_{sv} = K_b$ . For the system described above,  $K_b = 3.2 \times 10^4 \text{ mL/g}$ . Dividing  $K_b$  by the fraction of organic carbon in the sorbent yields a  $K_{oc}$  value of  $7.7 \times 10^4 \text{ mL/g}$  where  $K_{oc}$  is the equilibrium association constant normalized to organic carbon content. This value is well within the range of values reported by other researchers for the association of PAH to DOM. Using the fluorescence quenching technique to follow anthracene binding to a variety of humic materials,  $K_{oc}$  values varied from  $1-13 \times 10^4 \text{ mL/g}$ . Landrum (1984) reported values of  $1-8 \times 10^4 \text{ mL/g}$  for anthracene binding to humic material in aqueous solution using a reversed-phase method, and values of  $3-50 \times 10^4 \text{ mL/g}$  using a dialysis technique.

Wijayarathne and Means (1984b) reported a value of  $51 \times 10^4$  mL/g for anthracene binding to estuarine colloids.

In view of this agreement with other methods, we believe that the Stern-Volmer quenching constants are best interpreted as ground state association constants and not as bimolecular quenching rate constants. The same conclusion was drawn by Patonay et al. (1985) who used fluorescence quenching to follow the interaction between pyrene and cyclodextrin.

The fact that Stern-Volmer plots are linear allowed us to rule out other possible mechanisms. If two fluorophors are present and one is not accessible to quencher, the plot will deviate from linearity towards the x axis. If the quenching process is actually a combination of both static and dynamic quenching then the plot deviates from linearity towards the y axis (Lakowicz, 1983). The possibility that PAHs bound to humics also fluoresce but with a lower efficiency was also considered. However, this too would produce a nonlinear Stern-Volmer plot.

Table 4 illustrates the variation of  $K_{oc}$  values for the interaction of pyrene, anthracene and phenanthrene with SF-1 and SF-2 fulvic acids and SH-4 humic acid under identical conditions. In all cases, the interaction of humic or fulvic acid with the three PAHs studied resulted in linear Stern-Volmer plots after correcting for inner filter effects. The percent relative standard deviations of the

TABLE 4.  $K_{oc}$  Values for the Interaction of Pyrene, Anthracene, and Phenanthrene with SH-4, SF-1, and SF-2 Humic Materials.

HUMIC MATERIAL	$K_{oc} \times 10^{-4}$ (mL/g)		
	PYRENE	ANTHRACENE	PHENANTHRENE
SH-4	16.1 (3.2)	13.0 (1.6)	8.5 (2.6)
SF-1	11.7 (1.9)	9.2 (0.9)	7.4 (0.8)
SF-2	5.4 (1.0)	4.5 (1.6)	2.4 (2.2)

The numbers in parenthesis are the percent relative standard deviations of the slopes of the Stern Volmer plots used to determine  $K_{oc}$ . They are cited to show how closely the plots fit the straight line model. They should not be interpreted as the precision to which  $K_{oc}$  can be measured.

slopes of the Stern Volmer plots are given in parenthesis to show how closely the plots fit the straight line model.

For a given humic material, pyrene binds more strongly than anthracene which in turn binds more strongly than phenanthrene. This order reflects the order of decreasing octanol-water partition coefficients for the three PAHs (Table 2). However, PAH solubility data does not correlate as well. Furthermore, for a given PAH, measured  $K_{oc}$  values varied by as much as a factor of 3 depending upon the source of the humic or fulvic acid. For the three humic materials considered here, the humic acid produced the largest interaction and the SF-2 fulvic acid resulted in the smallest interaction.

#### Ionic Strength and pH Effects

Pyrene  $K_{oc}$  values for a given humic material remained virtually constant over the environmentally important pH range of 5 to 8; however, they varied in a complex manner as a function of ionic strength. The greatest changes were found to occur in the region of low ionic strength. The effect of varying the ionic strength from 0.0 to 0.1M on the magnitude of pyrene-SH-4  $K_{oc}$  values is illustrated in Table 5 and Figure 5. In this experiment the ionic strength was estimated from the concentration of sodium acetate used to buffer the solution. An ionic strength of 0.0 corresponds to no buffer added to the solution. Pyrene  $K_{oc}$  values were largest when no

Table 5. Variations in Measured Pyrene-SH-4  $K_{oc}$  as a Function of Acetate Buffer Concentration.

[Sodium Acetate] (M)	$K_{oc} \times 10^{-4}$ (mL/g)
0.0	27.8
0.0001	20.8
0.001	17.9
0.01	16.1
0.1	13.6



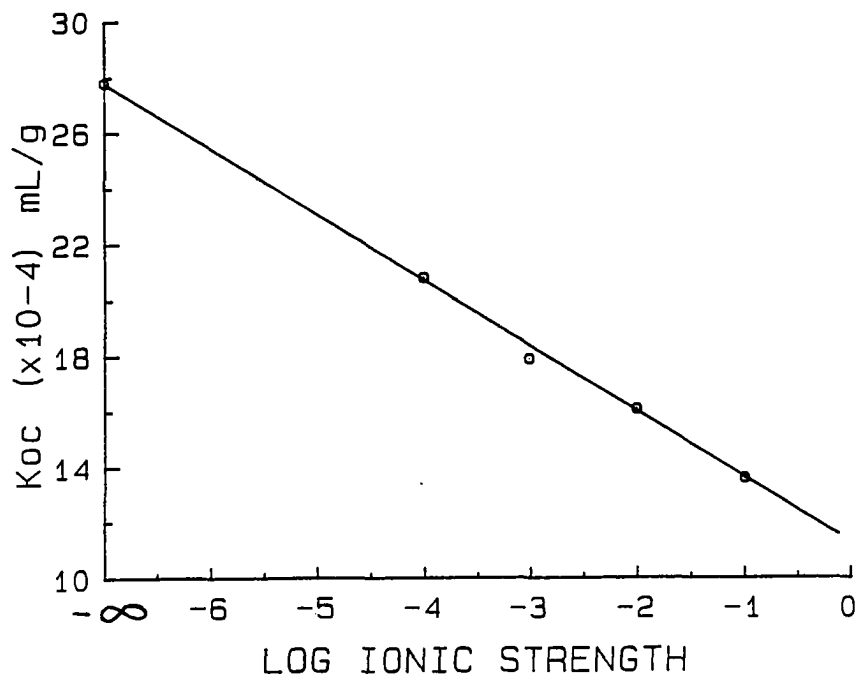


Figure 5. Variation in Measured Pyrene - SH-4  $K_{oc}$  as a Function of Log Ionic Strength Associated with Acetate Buffer Concentration.

buffer was added to the solution. As the concentration of added buffer increased,  $K_{oc}$  values decreased.

Solutions with an ionic strength greater than 0.01M were achieved by adding the appropriate amount of sodium chloride or calcium chloride to buffered, aqueous PAH solutions. At higher ionic strengths, the overall change in measured  $K_{oc}$  values was relatively small. The addition of calcium chloride resulted in a further decrease in  $K_{oc}$  beyond that noted at an ionic strength of 0.01M. However, as the concentration of sodium chloride was increased beyond 0.01M, the measured  $K_{oc}$  first decreased and then increased suggesting that  $K_{oc}$  varies in a complex fashion with ionic strength (Table 6).

In a factorial experiment designed to test the effects of both pH and ionic strength (added sodium chloride) on the interaction between pyrene and SH-2 humic acid, the pH was varied from 5.2 to 8.1 and the ionic strength ranged from a low of 0.01M (determined by the concentration of buffer) to a high value of 0.5M (determined by sodium chloride). The results are found in Table 7 along with the analysis of variance (ANOVA) table. An increase in  $K_{oc}$  was observed with increasing ionic strength, although the increase was not significant at the 95% probability level. This possible  $K_{oc}$  increase is consistent with the previous observations for sodium chloride solutions. There was no effect of pH in the range of 5 to 8 and no significant interaction between effects.

Table 6. The Effect of Ionic Strength (added NaCl and added CaCl<sub>2</sub>) on Measured K<sub>oc</sub> for the Interaction of Pyrene with SH-4 Humic Acid.

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Ionic Strength	K <sub>oc</sub> x10 <sup>-4</sup> (mL/g)	
	NaCl	CaCl <sub>2</sub>
0.01	16.1, 16.6	16.1, 16.6
0.1	12.8, 12.0	12.0, 12.4
0.5	14.6, 15.5	10.9, ----

---

Table 7a. Effect of pH and Ionic Strength on the Interaction of Pyrene with SH-2 Humic Acid.

X1 Ionic Strength, (M)	X2 pH	$K_{oc}$ x10 <sup>-4</sup> (mL/g)
-1 (0.01)	-1 (5.2)	6.4, 6.1
-1 (0.01)	+1 (8.1)	6.4, 5.4
+1 (0.51)	-1 (5.2)	9.5, 6.9
+1 (0.51)	+1 (8.1)	9.0, 5.9
0 (0.25)	0 (6.6)	8.5, 7.7

Table 7b. ANOVA for the Factorial Experiment Displayed in Table 7a.

Factor	Effect(x10 <sup>-4</sup> )	Mean Square	F Ratio
X1	+1.75	6.125	3.38
X2	-0.55	0.605	0.33
X1X2	-0.20	0.08	0.04
Lack of Fit		4.116	2.27
Error		1.81	

$$F_{.95} (1,5) = 6.61$$

Two additional experiments were conducted in which the ionic strength was varied from 0.003M (estimated from the concentration of phosphate buffer) to 0.51M through the addition of sodium chloride. In one case the ionic strength of the stock humic acid solution was not adjusted with sodium chloride and only the ionic strength of the PAH solution was varied. In the second experiment both the stock humic acid solution and the PAH solution were adjusted to the appropriate ionic strength. In addition the stock humic acid solution was allowed to age for 24 hours. The results of both experiments reveal that small additions of sodium chloride produce a decrease in measured  $K_{oc}$  up to an ionic strength of approximately 0.1M. Subsequent additions of sodium chloride beyond an ionic strength of 0.1M cause an increase in measured  $K_{oc}$  (Figure 6).

Several researchers have reported conflicting results for the effect of ionic strength on the sorption of hydrophobic organic pollutants to DOM. Carter and Suffett (1982) found slight increases in the amount of DDT bound to dissolved humic acid upon increasing the ionic strength from 0.001 to 0.08M. Similarly, Diachenko (1982) reported an increase in the amount of HCBd sorbed to humic acid as the ionic strength was increased from 0.0 to 1.0. However, for the sorption of linuron and atrazine by natural colloids, Means and Wijayarathne found that  $K_{oc}$  values increased when the salinity was decreased by a factor of 2.

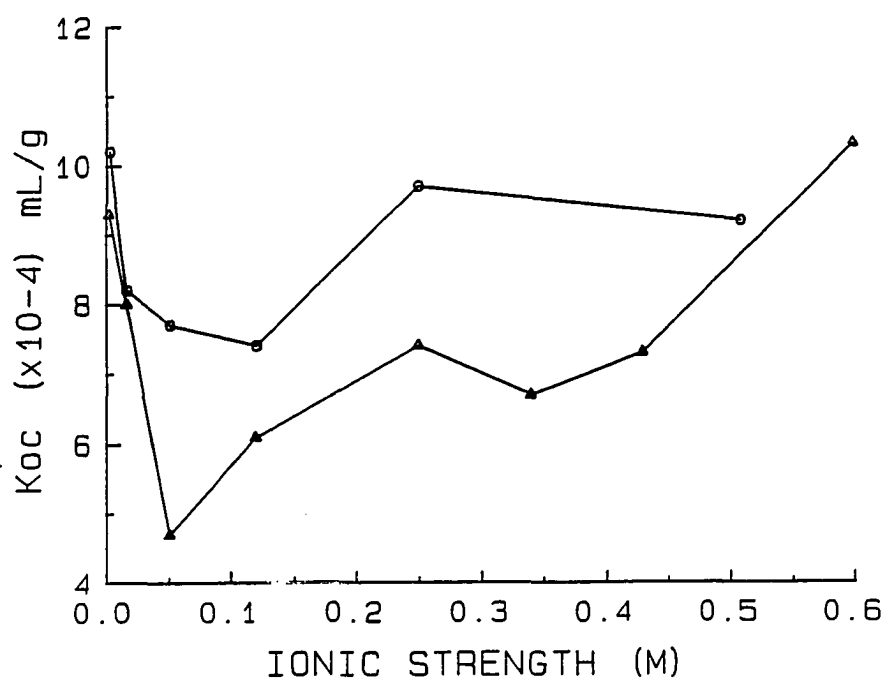


Figure 6. Effect of Ionic Strength on the Interaction of Pyrene with SH-2 Humic Acid ( $\circ$ ) and SH-2 Humic Acid 'Aged' 24 Hours ( $\Delta$ ).

Delaney (1985) investigated the effects of humic acid agglomeration on the sorption of pyrene. Ferric ion was shown to agglomerate dissolved humic acids and to alter their affinities for pyrene. In the presence of ferric ion, an increase in ionic strength through the addition of sodium chloride resulted in a measurable decrease in  $K_{oc}$ . In the absence of ferric ion, an increase in ionic strength resulted in no significant changes in  $K_{oc}$ . It should be noted that Delaney used a  $2^2 + 1$  design in which  $K_{oc}$  values were measured at ionic strengths of 0.01 and 0.5 and thus complex behavior would not be detected.

Some authors have attributed ionic strength effects to changes occurring in the polymeric structure of the humic material (Carter and Suffett, 1982; Means and Wijayarathne, 1982; Delaney, 1985). The Fuoss Effect describes "the coiling of a molecule as a result of the neutralization of its negatively charged functional groups, rendering the molecule both smaller and more hydrophobic" (Randtke and Jepsen, 1982; Ong and Bisque, 1968). Depending upon the mechanism of sorption, this may produce decreased sorption due to a reduction in the size of the molecule or increased sorption due to an increase in hydrophobicity of the molecule. Others have invoked salting out effects to explain an increase in sorption as the ionic strength is increased. At high ionic strengths the solubility of the pollutant decreases resulting in increased sorption.

The results reported here suggest that two effects may

be operating. At low ionic strengths (0.0 - 0.1M) it is believed that large changes in polymer conformation occur while changes in PAH solubility are small. Whitehouse (1984) examined the effect of salinity on PAH solubility and found no significant difference in solubility between distilled water and 4 ‰ seawater or between 33 ‰ and 36 ‰ seawater. He added that where salting out was observed, fairly large changes in salinity were required to produce significant changes in solubility. We believe that in the low ionic strength region, the Fuoss Effect dominates and sorption is reduced due to a decrease in the size of the humic acid molecule. At an ionic strength near 0.1M, the humic polymer may be fully contracted so that any additional increase in ionic strength only serves to decrease the solubility of the pollutant thereby increasing sorption.

When the stock humic acid solution was allowed to age 24 hours in the presence of sodium chloride,  $K_{oc}$  values were reduced further suggesting that conformational changes in the polymer may take days or longer to reach equilibrium. Delaney (1985) also noted the importance of 'aging' humic acid solutions. Both plots in Figure 6 appear to reach a minimum value at an ionic strength of 0.1M which may be the ionic strength at which maximum contraction takes place for SH-2 humic acid. This value is most likely determined by a number of factors including the molecular weight and total acidity of the polymer.



Based on the results for the SH-4 humic acid, it appears that the  $K_{OC}$  values also reach a minimum at an ionic strength of 0.1M. However, when calcium chloride is the ionic strength determining species, the  $K_{OC}$  values continue to decrease for ionic strengths  $> 0.1M$ , though slightly. The effect of cations on humic and fulvic acids has been examined in the past. Ong and Bisque (1968) reported that trivalent cations are more effective in coagulating humic matter than divalent cations and divalent cations are more effective than monovalent cations. They also investigated the coagulating ability of a series of monovalent cations and found small differences in the effectiveness of coagulation. The order of increasing coagulating power was  $K > Na > Li$  indicating that the cation with the largest ionic radius within a common valence is the most effective coagulant. This result may explain why  $K_{OC}$  values varied slightly as a function of buffer solution composition though the ionic strength and pH remained the same. This was found to be the case in this study and also by Delaney (1985).

#### **Agglomeration Effects**

Throughout the course of this investigation, fresh humic and fulvic acid solutions were prepared periodically due to eventual bacterial growth in old solutions. Stock solutions were prepared at concentrations of 500 ppm or 100 ppm and added to PAH solutions in 10 uL or 50 uL aliquots so

as to achieve similar final humic acid concentrations. For experiments that were duplicated in all respects except for the concentration of stock humic acid solution, it was noted that  $K_{oc}$  values varied in a systematic way (Table 8). When aliquots were taken from a 500 ppm stock solution,  $K_{oc}$  values were a factor of 3 larger than when aliquots were taken from a 100 ppm stock solution. The reason for this is uncertain; however, it is believed that these differences may be due to humic acid agglomeration effects. After this effect was noted, all  $K_{oc}$  estimates were made using a 100 ppm stock humic acid solution to avoid errors from this source.

The polydispersity, or state of agglomeration, of humic materials is known to vary as a function of concentration (Lochmuller and Saavedra, 1986; Stevenson, 1982). Several researchers have noted an effect of DOM concentration on pollutant binding affinity (McCarthy and Jimenez, 1985a; Hasett and Millicic, 1985; Landrum et al., 1984; and Carter and Suffett, 1982). Each found slight decreases in the magnitude of the association constant as the concentration of DOM was increased which is opposite in direction to the effect noted here. Perhaps the largest decrease was reported by Landrum using a reversed-phase separation procedure. Measured  $K_{oc}$  values decreased by a factor of 3 over a DOM concentration range of 2 - 16 mg/L. In this study, using the reversed-phase procedure to measure the binding of anthracene to Aldrich humic acid, measured

Table 8. Variations in Measured  $K_{oc}$   $\times 10^{-4}$  (mL/g) as a Function of Concentration of Stock Humic Acid Solution.

System	Stock Humic Acid Solution	
	500 ppm	100 ppm
Anth-SF-1	9.2	3.2
Anth-SF-2	4.5	1.6
Anth-SH-4	13.0	3.7
Pyrene-SF-1	11.7	4.3
Pyrene-SH-4	11.5	4.5

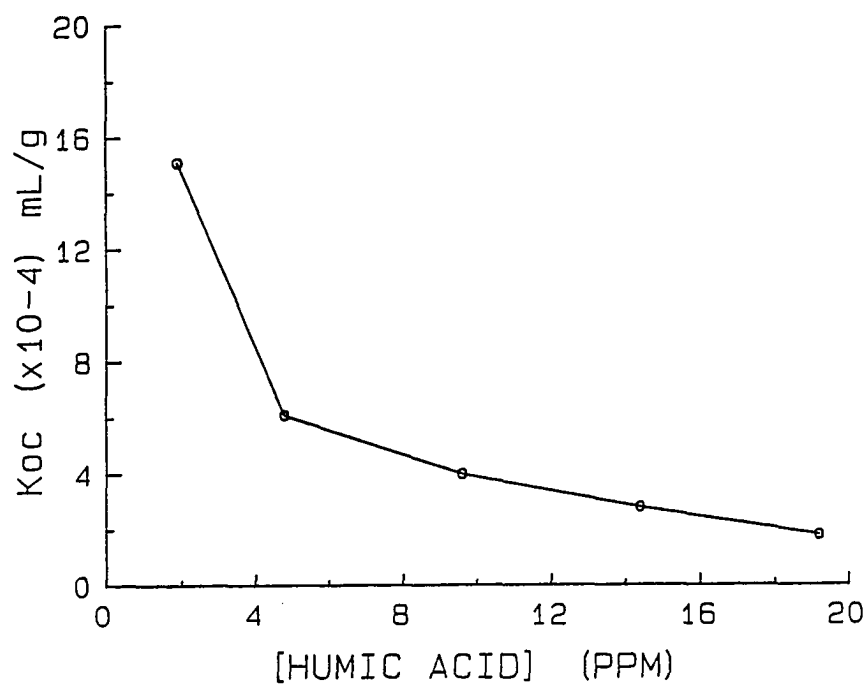


Figure 7. Effect of Humic Acid Concentration on Measured  $K_{oc}$  Values Using a Reversed-Phase Procedure.

$K_{oc}$  values decreased by a factor of 8 over a humic acid concentration range of 2 - 19 mg/L (Figure 7). Using fluorescence quenching titrations to determine  $K_{oc}$  values, linear Stern Volmer plots were found in all cases indicating that there was no effect of humic acid concentration changes in the 0-20 mg/L range on  $K_{oc}$  estimates. Instead, differences observed for the initial stock humic acid solutions suggest that it is the polydispersity of the humic material rather than the concentration per se that controls binding. If the kinetics of disaggregation are slow (i.e. longer than the time allowed for equilibration to take place in the fluorescence quenching titrations), then by adding humic acid from stock solutions of different concentrations one may be adding humic acid in different states of agglomeration even though final concentrations may be the same. Regarding the determination of  $K_{oc}$  values using the reversed-phase procedure, it is believed that the increased agglomeration of humic acid at higher concentrations may result in some material being retained by the Sep-Pak cartridge. This action would result in an underestimation of bound PAH and consequently an underestimation of the  $K_{oc}$  value.

Chiou et al. (1986) investigated the water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. In that study they speculate that a partition like interaction of the solute with the "microscopic organic environment" of dissolved

humic molecules would be a reasonable explanation for solute solubility enhancement. Furthermore, if this is true then the effectiveness of such an interaction would depend on the size and polarity of the humic molecule; i.e. the humic molecule must be sufficiently large and possess a sizeable intramolecular nonpolar environment to promote solute partitioning. Since this is indeed what they found, it provides a rational explanation for the results found here. The 500 mg/L stock humic acid solution may promote a higher degree of agglomeration compared to the 100 mg/L stock solution and as a result provide a better environment for binding PAH. Delaney (1985) reported similar findings. He used light scattering to follow the complexation of ferric ion by humic acid and evaluated resultant conformational changes. The addition of ferric ion to aqueous humic acid solutions caused humic acid to agglomerate. Subsequent fluorescence quenching studies using the agglomerated humic acid solutions showed that pyrene was more strongly bound by humic acids in a greater state of agglomeration.

#### **Reproducibility**

Using the fluorescence quenching technique,  $K_{oc}$  values were determined with a relative standard deviation of 3.8% (%RSD). This estimate was determined from 20 duplicate measurements taken over a period of 3 years and under a variety of solution conditions. The data in Table 7 illustrates that reproducibility is better for solutions of

low ionic strength. The larger variations at high ionic strengths could be due to the fact that the humic acid may not have reached its equilibrium conformation. Delaney reported a reproducibility of 3.3 % (%RSD) using the same technique.

#### Comparison Study

Since measured  $K_{oc}$  values were found to vary in a complex manner as a function of the humic material used, its aggregation, and the ionic strength, a method comparison study was undertaken in which these variables were controlled. The fluorescence quenching technique was compared to the reversed-phase separation technique described by Landrum et al.(1984).  $K_{oc}$  values for the interaction of anthracene with 5 different humic and fulvic acids were determined using both methods under the same pH and ionic strength conditions and the same stock humic acid solutions (Table 9). Listed under the heading 'reversed-phase' are  $K_{oc}$  determinations at four different concentrations of humic or fulvic acid. The  $K_{oc}$  values are listed in the order of increasing DOM concentration from left to right.

It is difficult to make a direct comparison between the two methods since the reversed-phase method is subject to DOM concentration effects as noted earlier. However, if  $K_{oc}$  values really were changing, this would be reflected in nonlinear Stern-Volmer plots which are not found.

Table 9. Comparison of  $K_{oc}$  Values Determined by Fluorescence Quenching and a Reversed-Phase Separation Technique.

	$K_{oc} \times 10^{-4}$ (mL/g)	
	Fl. Quenching <sup>a</sup>	reversed-phase <sup>b</sup>
SF-2	1.6	3.8, 1.9, 1.0, 1.1
SF-1	3.2	5.7, 2.9, 1.9, 1.6
Ald	5.2	6.1, 4.0, 2.8, 1.8
SH-4	3.7	3.6, 1.8, 1.4, 1.1
SH-5	6.4	5.3, 2.6, 2.4, 2.3

<sup>a</sup> the concentration range extends from 0-9 ppm except for the SW FA which is from 0-17 ppm.  
<sup>b</sup> the four values correspond to four different humic or fulvic acid concentrations; 4.8, 9.6, 14.4, 19.2 ppm except for the SF-1 where concentrations were 6.0, 11.9, 17.9, 23.8 ppm.



Therefore we believe that variations in  $K_{oc}$  are caused by chromatographic effects due to changes in agglomeration.

In general, despite DOM concentration effects using the reversed-phase procedure, the two methods compare very favorably. At a DOM concentration of 4.8 ppm,  $K_{oc}$  values by the Landrum procedure for the three humic materials are in excellent agreement with fluorescence quenching values, while estimates for the two fulvic acids differ by a factor of two. At the 4.8 ppm concentration, the correlation coefficient for fluorescence quenching and reversed-phase values is low ( $r = 0.57$ ) primarily due to the poor agreement of the fulvic acids. At a concentration of 19.2 ppm, the fluorescence quenching results exceed those determined by the reversed-phase method by a factor of two for the fulvic acids and a factor of three for the humic acids. The correlation coefficient, however, increases to a value of 0.87.

#### **DOM Effects**

Throughout the course of this study, pyrene  $K_{oc}$  estimates were obtained for 16 different humic and fulvic acids. Measured  $K_{oc}$  values varied by a factor of 10 depending upon the humic material. For this reason it was of interest to determine what structural or functional characteristics of the humic materials were important in binding pyrene. We are aware of only one study which has related spectral characteristics of humic materials with

organic pollutant sorption data. Diachenko (1981) used cross polarization magic angle spinning (CPMAS)  $^{13}\text{C}$  NMR, infrared analysis and elemental analysis to correlate chemical and spectral properties of three different humic materials to their hexachloro-1,3-butadiene (HCBd) adsorption affinities. The correlations indicate that HCBd was preferentially adsorbed to humics rich in hydrophobic paraffinic components. Results in our lab revealed that PAHs were bound more strongly to humic materials possessing a high degree of aromaticity.

Presented here are  $^{13}\text{C}$  solid state NMR, infrared analysis, UV absorbance data and elemental analysis for humic and fulvic acids from several sources. All humic materials were characterized with respect to elemental analysis and infrared and UV absorbance. In addition, four of the humic acids (MH-5, SH-3, SH-4, and SH-5) were examined using  $^{13}\text{C}$  solid state NMR. They were chosen because they represent the greatest variations in terms of pyrene binding capacity, UV and infrared absorbance, and elemental analysis as well as because they were available in large enough amounts to obtain the NMR spectra. As a result, comprehensive spectral analysis of only these four humic acids will be discussed in detail.

The infrared spectra of the four humic materials illustrated in Figure 8 show characteristically broad absorption bands but they were of equal or better quality than previously reported spectra in the literature. All four

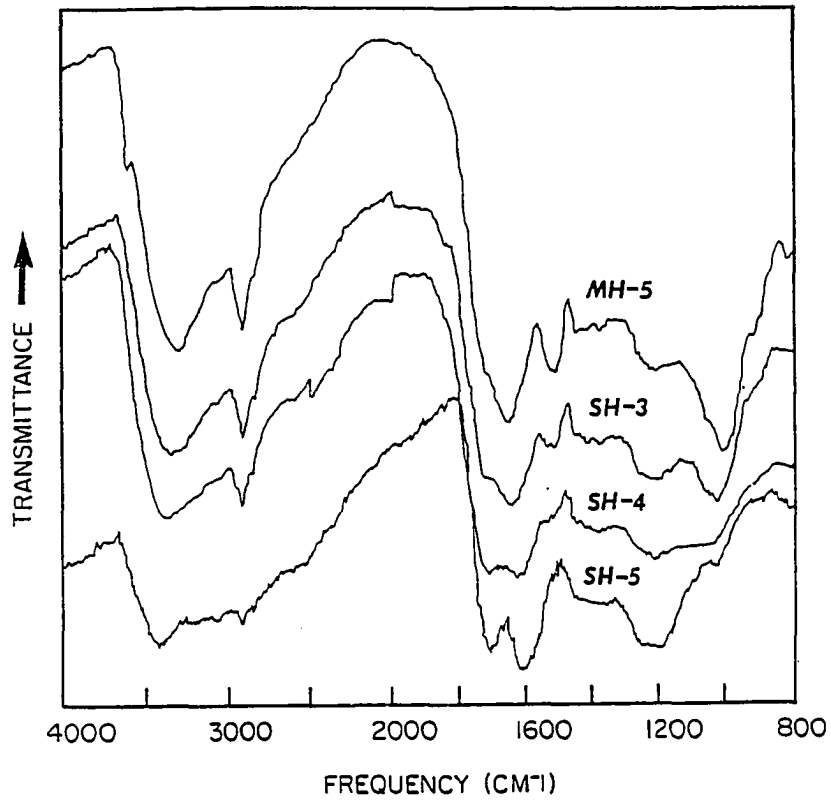


Figure 8. Infrared Spectra of Humic Acid Samples MH-5, SH-3, SH-4, and SH-5.

spectra exhibited a rather strong and broad band in the region of  $3400\text{ cm}^{-1}$ . According to Stevenson and Goh (1971), this band probably arises from H-bonded OH groups including those of COOH or possibly N-H groups. The band is strongest for the MH-5 humic acid and decreases in intensity in the order MH-5 > SH-3 > SH-4 > SH-5. The next region of interest is at  $2910\text{ cm}^{-1}$ . This absorption band is observed in all four spectra and is due to aliphatic C-H stretching. It is interesting to note that the order of decreasing intensity for this peak parallels the trend seen for the peak at  $3400\text{ cm}^{-1}$ .

Major differences occur in the region of  $1750\text{--}1500\text{ cm}^{-1}$ . The MH-5 sample exhibits bands at  $1520$  and  $1650\text{ cm}^{-1}$  with a very slight shoulder at  $1720\text{ cm}^{-1}$ . For the SH-3 sample, the peaks at  $1520$  and  $1650\text{ cm}^{-1}$  are diminished in size and the shoulder at  $1720\text{ cm}^{-1}$  is more pronounced. For the SH-4 humic acid we find that the band at  $1520\text{ cm}^{-1}$  is further diminished in size, the band at  $1650$  has shifted to  $1620\text{ cm}^{-1}$  and the shoulder at  $1720\text{ cm}^{-1}$  has grown into a band almost equal in intensity to the band at  $1620\text{ cm}^{-1}$  and it is now centered at  $1705\text{ cm}^{-1}$ . In the SH-5 sample the bands at  $1520$  and  $1650\text{ cm}^{-1}$  have disappeared and instead we find two strong bands centered at  $1700$  and  $1605\text{ cm}^{-1}$ .

The bands at  $1520$  and  $1650\text{ cm}^{-1}$  may be attributed to the amide I and amide II bands of polypeptide linkages from the reaction of amino acids. If this is the case then

the absorption at  $3400\text{ cm}^{-1}$  may in fact be due to N-H stretching. Atomic N/C ratios are found in Table I and correlate well with these absorptions suggesting that this may be a valid conclusion.

The shoulder at  $1720\text{ cm}^{-1}$  that grows into a band for the SH-4 and SH-5 humic acids is most likely due to the C=O stretch of COOH. This suggests that the marine humic acid, MH-5, is less acidic than the soil humic acid SH-5. Others have also noted that humic acids from well developed soils possess higher acidity than do marine humics (Rashid and King, 1970).

Tentative peak assignments for the major absorptions found in the region of  $1720\text{--}1500\text{ cm}^{-1}$  are listed in Table 10. Based on these assignments it appears that the COOH groups are most likely attached to aromatic carbons since the observed increase in peak intensity at  $1720\text{ cm}^{-1}$  is accompanied by a decrease in the size of the C-H stretching frequency at  $2920\text{ cm}^{-1}$ .

The only other band that may be of importance is centered at  $1015\text{ cm}^{-1}$ . The band is strongest in the MH-5 sample, is diminished in the SH-3 sample and is practically absent in the SH-4 and SH-5 samples. Possible assignments might be Si-O of silicate impurities, C-O stretching of polysaccharide type structures, or C-H parallel bend in an aromatic ring. The disappearance of this band parallels the disappearance of bands at  $1520$  and  $1650\text{ cm}^{-1}$ .

Table 10. Tentative Peak Assignments for Major Absorptions Found in the Region of 1720-1500  $\text{cm}^{-1}$  of the Infrared Spectra of Humic Materials.

Wavenumber ( $\text{cm}^{-1}$ )	Tentative Assignment
1520	polypeptide amide II band (N-H bending)
1600	aromatic C=C, C=O of quinones or conjugated ketones
1650	polypeptide amide I band (C=O stretch)
1720	C=O of carboxylic acids

Solid state  $^{13}\text{C}$  NMR spectra of each of the humic acids are depicted in Figure 9. Six major resonances are evident. As previously noted there exists a relatively narrow strong absorbance in the region of 35 ppm. This band is strongest in the MH-5 sample and of about equal intensity in the other three samples. The narrowness of the band suggests the presence of a repeating structural unit which is usually characteristic of long chain aliphatic hydrocarbons.

The region of 50-90 ppm has been attributed to aliphatic carbon singly bonded to one oxygen atom. In these spectra this region shows two discernable absorptions: from 50-70 ppm represents methoxyl carbons while the band from 70-90 ppm can most likely be attributed to OH substituted and ether bound aliphatic carbons as well as ring carbons of carbohydrates. The peak at 50 ppm is almost absent in the SH-5 sample and then appears to increase in size on going from SH-5 < SH-4 < SH-3 < MH-5.

The next area of interest is in the region of 100-150 ppm. Resonance in this region is attributed to aromatic and unsaturated carbon atoms. A number of investigators have integrated the area under the curve in this region and divided that value by the total integrated area to calculate the fraction of aromatic carbon atoms ( $f_a$ ) in the sample (Thurman and Malcolm, 1983).  $f_a$  values for the four samples presented here are found in Table 11. For several reasons relating to the method of integration and the absence of

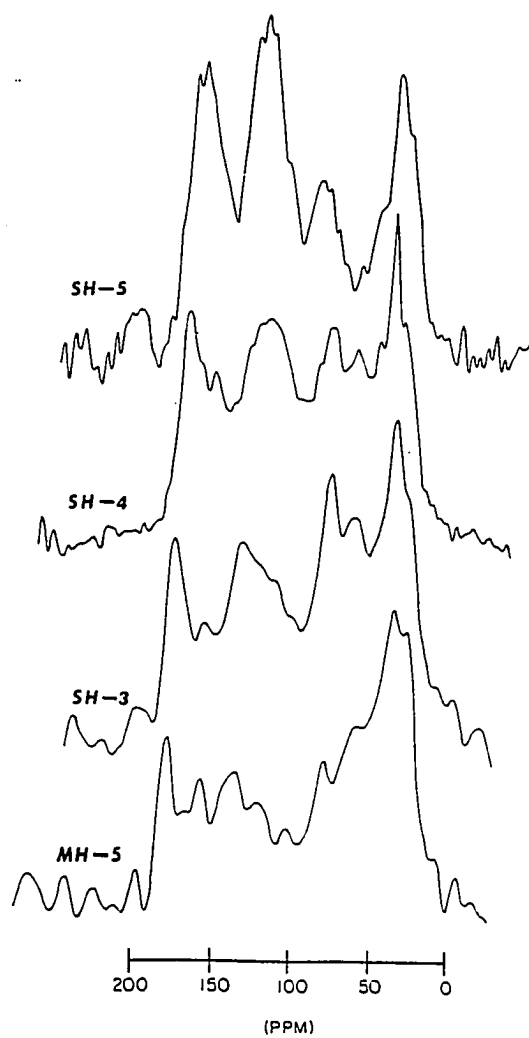


Figure 9.  $^{13}\text{C}$  Solid State NMR Spectra of Humic Acid Samples MH-5, SH-3, SH-4, and SH-5.



TABLE 11. Fraction of Aromatic Carbon (fa) and Fraction of Carboxylic Acid Carbon (fCOOH) Determined by  $^{13}\text{C}$  Solid State NMR.

Humic Material	fa	fCOOH
MH-5	20	9
SH-2	24	11
SH-3	28	14
SH-5	34	20

standards, we believe that these estimates may be somewhat low on an absolute basis, but the relative values are believed to be valid. Therefore correlations based on these values should be valid.

A small peak is visible in the MH-5, SH-3, and SH-4 samples in the region of 153-157 ppm. Resonances in this region have been attributed to phenolic carbon. It is interesting to note that this peak is absent in the SH-5 sample which is the richest in aromatic carbon. There is however a very large resonance at 168 ppm which may in fact be masking any resonance at 155 ppm.

The final region of interest is 160-190 ppm which has been attributed to carbon atoms doubly bonded to one oxygen atom, i.e., carboxylic acids, amides, or esters. In an analogous manner to the determination of the fraction of aromatic carbon, the fraction of carbon in the form of COOH ( $f_{\text{COOH}}$ ) can be calculated with the assumption that  $\text{NC}=\text{O}$ , and COOR represent a small fraction compared to COOH (this assumption may not be valid for the marine humic acid).  $f_{\text{COOH}}$  values are also found in Table 11. Interestingly the change in  $f_{\text{COOH}}$  parallels the change in the fraction of aromatic carbon.

$K_{\text{OC}}$  values were determined for the association of pyrene to each of the humic materials in the dissolved state. All experiments were buffered at a pH of 5.6 - 6.0 using a 0.01 M acetate buffer system. The results are found in Table 1 along with the elemental composition and UV

absorptivity for each of the humic materials.  $K_{oc}$  values varied by as much as an order of magnitude depending upon the origin of the humic material. With such large variations it was possible to critically evaluate the relationships of physical and spectral characteristics with changes in  $K_{oc}$  values.

Three independent measures of the relative degree of C=C bond formation each proved to be very highly correlated with pyrene  $K_{oc}$  values as shown in Figures 10-12. Correlation coefficients ranged from 0.69 to 0.997. The best correlation, illustrated in Figure 10, was the fraction of aromatic carbon as determined by solid state NMR where  $r = 0.997$  for the four humic acids studied. The utility of this technique seems unequivocal; however, as has been noted, its use is limited to the relatively few laboratories with access to the necessary equipment (Newman and Tate, 1984).

A more easily obtained experimental measure that still correlates well with  $K_{oc}$  values is the UV absorptivity taken at a wavelength of 272 nm. A wavelength of 272 nm was used because absorbance measurements were readily available from fluorescence quenching studies and 272 nm is in the region of  $\pi$ - $\pi^*$  transitions in substituted benzenes and most polyenes. However, adjacent wavelengths such as 254 nm should give an equally useful correlation. For the same four humic acids a correlation coefficient of 0.987 was obtained. Figure 11 illustrates the correlation between the

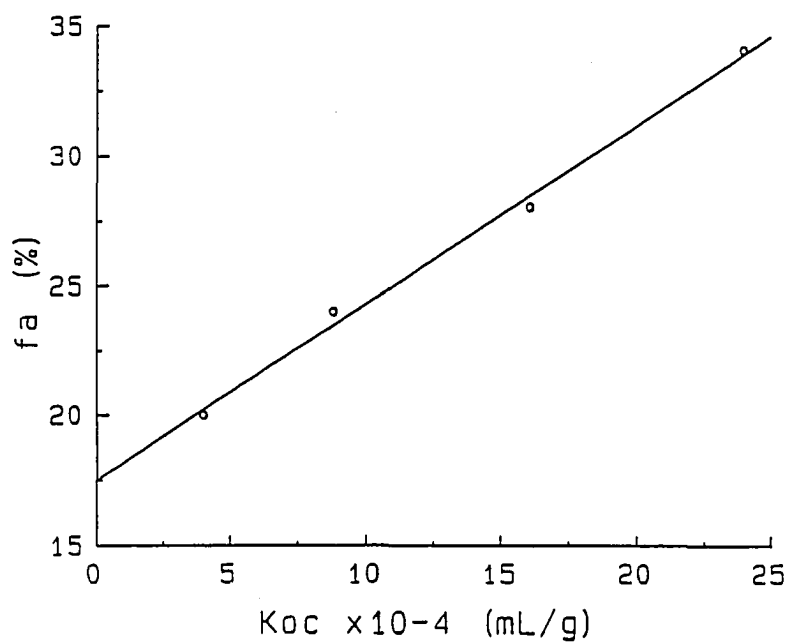


Figure 10. Correlation Between Pyrene  $K_{oc}$  Values and the Fraction of Aromatic Carbon in the Humic Acids as Determined by  $^{13}\text{C}$  NMR.

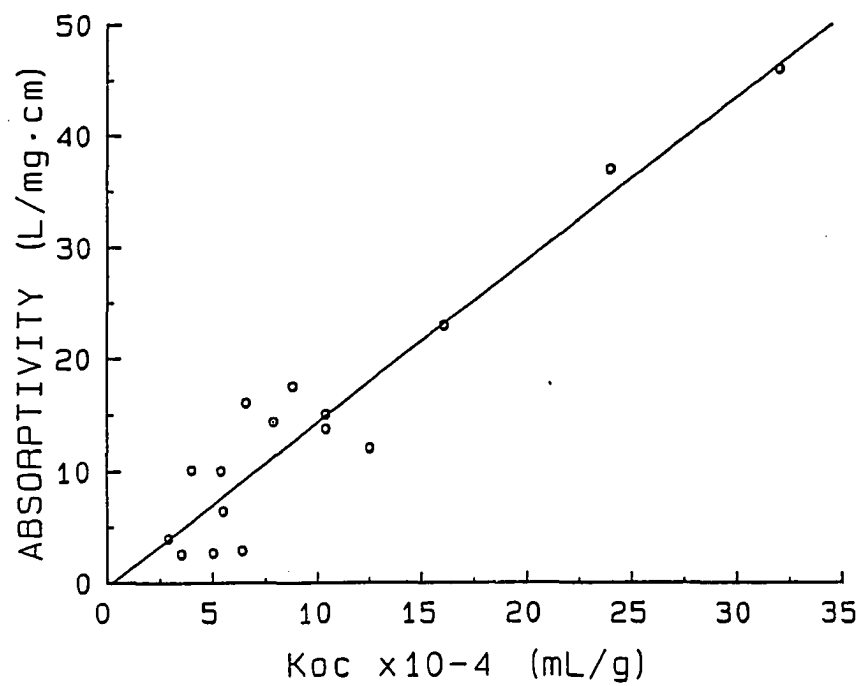


Figure 11. Correlation Between Pyrene  $K_{oc}$  Values and the Absorptivity at 272 nm of the Humic Materials.

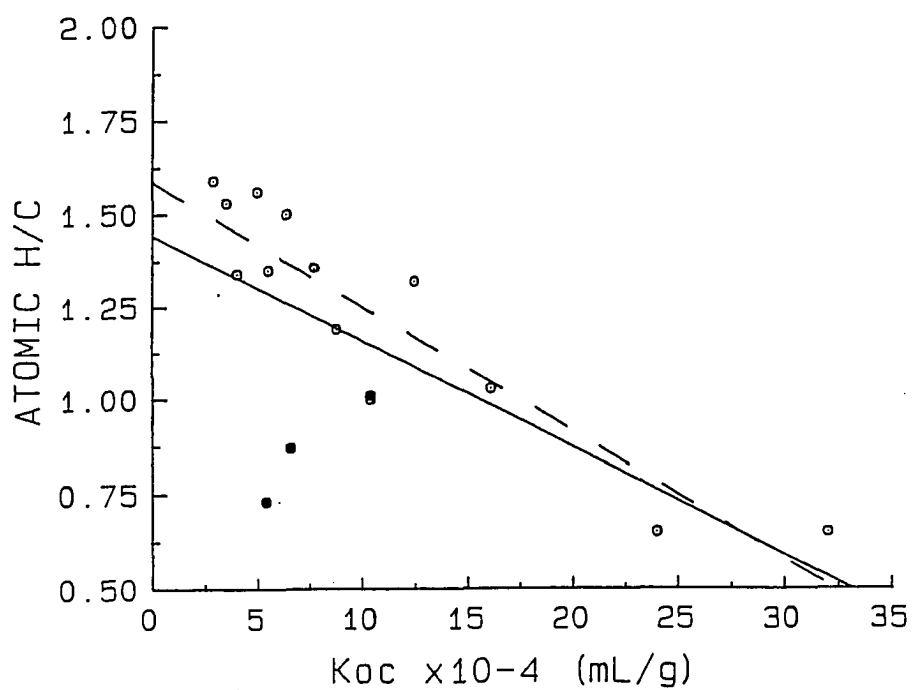


Figure 12. Correlation Between Pyrene  $K_{oc}$  Values and the H/C Ratio of the Humic Materials.

absorptivities taken at 272 nm and the pyrene  $K_{oc}$  values for the 13 humic acids and 3 fulvic acids shown in Table 1. The correlation remained very highly significant with  $r = 0.95$ . The correlation of atomic H/C with pyrene  $K_{oc}$  values for the four humic acids revealed a correlation coefficient of 0.985. Increasing the number of samples to 16 however, decreases the correlation coefficient to  $r = 0.69$ . If the three fulvic acid data points (solid circles) are omitted from the data set, the coefficient increases to a value of 0.92 (Figure 12).

The reason for the low correlation coefficient with the fulvic acids included becomes apparent if we examine their infrared spectra. Figure 13 illustrates the infrared spectra of the SF-1 and SF-2 soil fulvic acids. The peaks at 3400 and 2920  $\text{cm}^{-1}$  are very small and the region between 1720 and 1500  $\text{cm}^{-1}$  is dominated by two peaks centered at 1715 and 1615  $\text{cm}^{-1}$ . The peak at 1715  $\text{cm}^{-1}$  is much larger than the peak at 1615  $\text{cm}^{-1}$ . Based upon peak assignments made for the humic acids this suggests that the fulvic acids are made up of the same basic structure as the soil humic acids but are much more substituted with COOH groups. Since both COOH and  $-\text{HC}=\text{CH}-$  have the same atomic H/C ratio, the presence of a high concentration of COOH groups would adversely affect the use of atomic H/C as a measure of  $-\text{HC}=\text{CH}-$ .

The six marine humic acids exhibited very similar qualities. This fact is reflected in the atomic C/N ratios

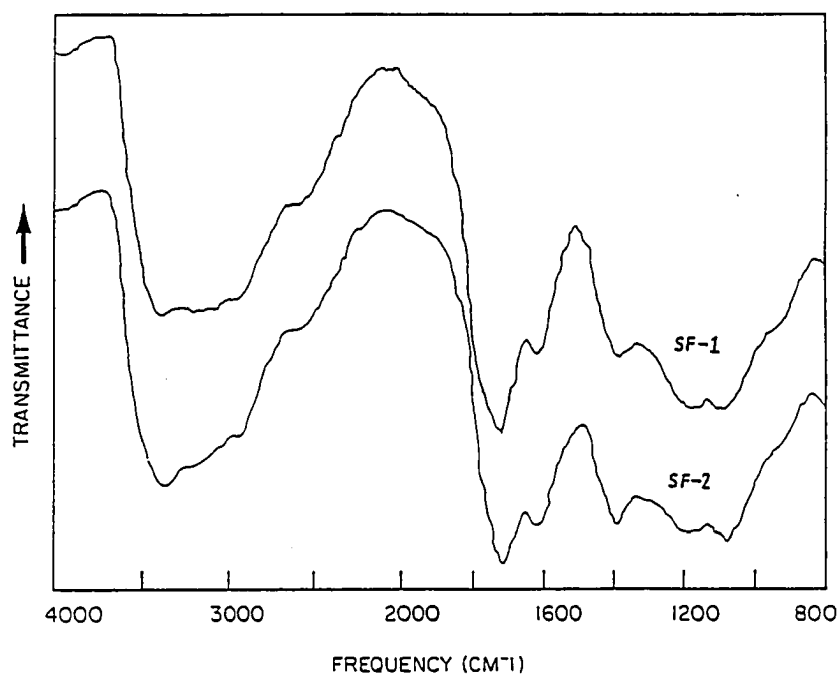


Figure 13. Infrared Spectra of the Fulvic Acid Samples SF-1 and SF-2.



which have been used in the past as source indicators for humic acids (Stuermer et al., 1978). In this study the marine humic acids exhibit a very small range of ratios varying from 7-10. In contrast, the ratios for the soil humics vary from 12-50 and the fulvic acid ratios vary from 75-107. One reason for this may be that the sampling of the marine and estuarine sediments involved only sampling the top 0-2 cm of sediment which excludes older subsurface humics. Conversely, the larger variation in soil humic acid ratios could be a result of the fact that humic acids of different ages were sampled. Jackson also noted a greater uniformity in marine humic substances (Jackson, 1975).

From this study it is clear that the affinity of dissolved humic materials for pyrene increases as the degree of aromaticity increases. This finding is in agreement with work done by Tanaka et al. (1982) on the effect of stationary phase structure on retention in reversed-phase liquid chromatography. They found that aromatic stationary phases showed greater retention for aromatic solutes and less retention for saturated hydrocarbons than the saturated stationary phases. Stationary phase effects, such as steric recognition and pi-pi interactions between solute and stationary phase, were found to be important in addition to solvophobic interactions. With respect to pollutant-DOM interactions it is important to stress that while the degree of aromatic character may be important for planar, aromatic PAHs, the degree of aliphatic character of the DOM

may be more important for nonplanar, saturated hydrocarbon solutes.

In terms of a binding mechanism for neutral PAH molecules, it is believed that binding is dominated by Van der Waals type interactions. Van der Waals forces are the attractive forces between instantaneous and induced dipole moments of molecules. The ease of inducing dipole moments depends upon the polarizability of the molecule. Just as molecular dipole moments can be estimated as the vector sum of bond moments, the molecular polarizability can be estimated as the sum of bond polarizabilities, some of which are listed below: (Levine, 1978)

<u>bond</u>	<u>C-C</u>	<u>C=C</u>	<u>C-H</u>	<u>C=O</u>	<u>C-Cl</u>
	0.64	1.66	0.65	1.16	2.61

It appears that an increase in the # of C=C bonds should increase the molecular polarizability and thereby increase the strength of the Van der Waals attraction forces. As a result, an increase in aromaticity of the dissolved humic materials may serve to increase the polarizability of the polymer and increase the strength of PAH binding. In addition PAHs are not very soluble and are subject to an additional "thermodynamic gradient" driving them out of solution (Voice and Weber, 1983). The combination of this driving force and Van der Waals forces is commonly referred to as "hydrophobic bonding".

The dependence of binding on the composition and structure of the humic material also has implications with respect to the formation of predictive models for determining the fate of pollutants in the environment. A number of models have been constructed in attempts to predict distribution coefficients to sediments and soils based on the aqueous solubility or octanol/water partition coefficients of solutes (Voice and Weber, 1983; Briggs, 1981). These models consider the quantity of carbon in the sorbent but not its 'quality' such as aromatic character. The fact that several investigators have generated different equations relating the same two parameters ( $K_{oc}$  vs.  $K_{ow}$ ) suggests that perhaps some centralizing parameter is absent in the equations. We believe that the 'quality' of the sorbent should also be considered in the design of environmental fate models for hydrophobic organic pollutants. This should be most important for solutes whose primary binding mechanism is through the formation of hydrophobic bonds.

#### **Application to Sediments**

Fluorescence quenching was also used to follow the sorption of anthracene to an estuarine sediment sample (OR sediment). On an organic carbon basis, measured  $K_{oc}$  values were similar for anthracene binding to OR sediment and anthracene binding to humic acid isolated from OR sediment (SH-2). However, OR sediment that had been

extracted with dilute base to remove a small portion of the humic materials was shown to bind anthracene more strongly than unaltered OR sediment.

The fluorescence quenching of anthracene in the presence of OR sediment is illustrated in Table 12 which includes the concentrations of sediment, absorbance readings at 250 nm and 380 nm, the fluorescence intensities corrected for dilution and inner filter effects, and calculated  $F_0/F$  values for each addition of titrant. The resulting Stern-Volmer plot is linear with a slope of 940 mL/g (Figure 14). Assuming that PAH sorption to sediments can be treated in the same manner as PAH sorption to DOM, the slope of the Stern-Volmer plot corresponds to an equilibrium association constant of 940 mL/g. When normalized to the fraction of organic carbon in the sediment, this constant converts to a  $K_{oc}$  value of  $3.6 \times 10^4$  mL/g. This value is very close to the  $K_{oc}$  value,  $2.8 \times 10^4$  mL/g, calculated for the association of anthracene to SH-2 humic acid (extracted from this sediment) under identical conditions. This similarity suggests that the humic acid associated with OR sediment is the primary constituent responsible for the sorption of anthracene to the sediment.

However, when OR sediment was extracted with dilute base to remove some of the organic matter, the anthracene  $K_{oc}$  value for the extracted sediment increased by a factor of 2 to  $6.6 \times 10^4$  mL/g (Figure 15). Carbon, hydrogen, and nitrogen analyses were performed on OR

Table 12. Fluorescence Quenching Titration Data for the Interaction of Radiolabelled Anthracene with OR Sediment.

---

[sediment]	A <sub>ex</sub>	A <sub>em</sub>	F <sub>obs.</sub>	F <sub>corr</sub>	F <sub>o</sub> /F
0	.038	.000	1783	1859	1.000
59	.042	.005	1644	1765	1.053
115	.045	.005	1542	1693	1.098
170	.052	.010	1413	1600	1.162
222	.059	.012	1320	1540	1.207
273	.066	.015	1236	1482	1.254

---

A<sub>ex</sub> and A<sub>em</sub> are absorbance values recorded at the exciting and emitting wavelengths.  
 F<sub>obs.</sub> is the observed fluorescence intensity.  
 F<sub>corr.</sub> is the fluorescence intensity corrected for inner filter and dilution effects.

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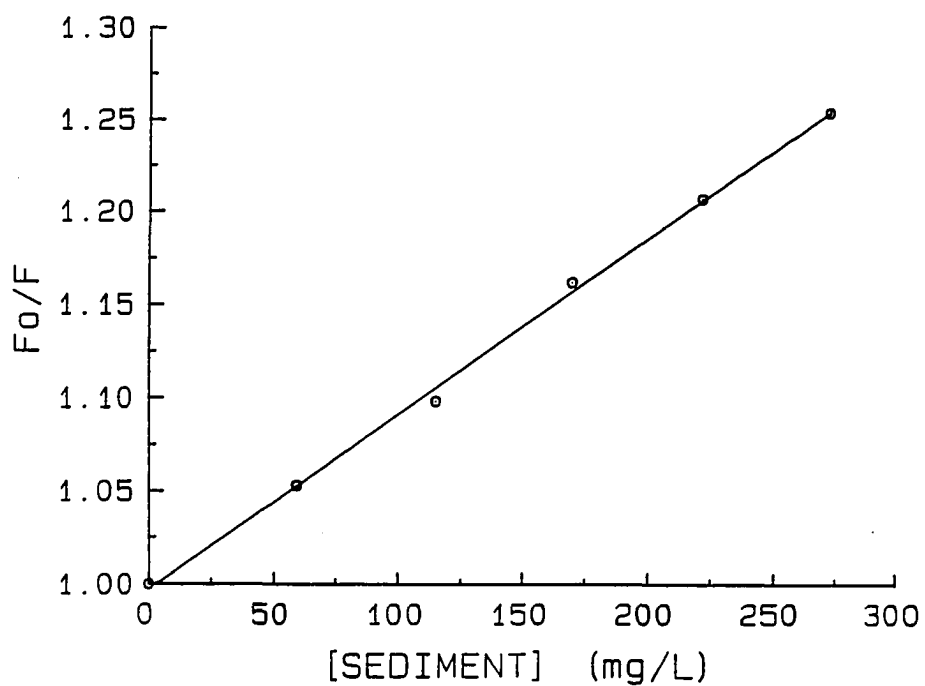


Figure 14. Stern-Volmer Plot for the Interaction of Anthracene with Unaltered OR Sediment.

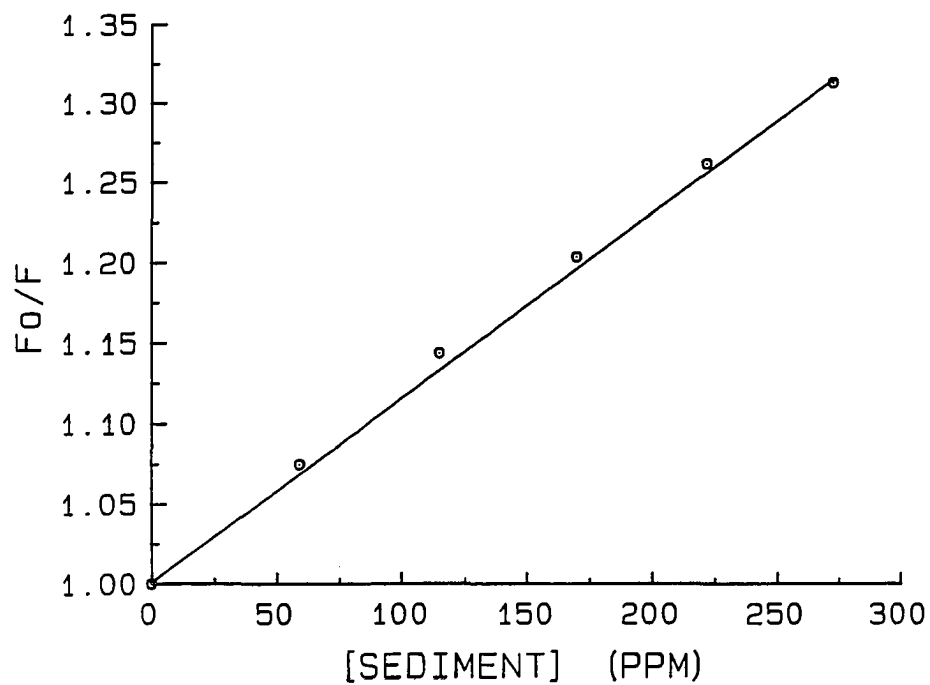


Figure 15. Stern-Volmer Plot for the Interaction of Anthracene with OR Sediment Extracted with Dilute Base.

sediment before and after extracting with base. The presence of inorganic carbon was not detected using 4N HCl and thus it was assumed that the measured carbon was organic carbon. After extracting with dilute base, the % carbon decreased from 2.58 % to 1.73 %; indicating that a significant amount of organic material still remained associated with the sediment. This material was most likely enriched in humin, the high molecular weight, highly condensed, acid and base insoluble fraction of humic material. It is believed that humin possesses the highest degree of aromaticity among the various fractions of humic material (Stevenson, 1982). Based on the previously established correlation of  $K_{oc}$  with aromaticity of the humic material, it is not surprising that anthracene binds more strongly to OR sediment that has been extracted with base.

A batch equilibration procedure using radiolabelled anthracene was also employed to estimate  $K_{oc}$  for the interaction between anthracene and OR sediment. Scintillation counting was used to quantitate the total amount of anthracene in solution, the amount of anthracene remaining in the supernatant after centrifuging for 45 minutes at 8800 rpm, and the amount of anthracene in the supernatant that passed thru a C-18 Sep-Pak cartridge. The Sep-Pak separation step represents an attempt to distinguish between freely-dissolved anthracene and anthracene bound to DOM. The amount of anthracene bound to sediments and the



amount of anthracene freely-dissolved in solution were obtained by difference.

Several researchers have found that at high sediment concentrations, a significant amount of organic matter may leave the surface of the sediments as microparticulates in the aqueous phase and complicate the  $K_{OC}$  measurement (Gschwend and Wu, 1985; Voice et al., 1983). This observation has been offered as an explanation of the dependence of equilibrium partitioning on solids concentration. For a large number of solutes, the observed  $K_{OC}$  value has been shown to decrease as the concentration of solids increases. In this study, 2 solid concentrations were used, 100 and 500 mg/L. Each experiment was run in duplicate (Table 13). Each measure of  $^{14}C$  radioactivity was the average of triplicate analyses corrected for background radiation.

Before comparing calculated  $K_{OC}$  values using this method with  $K_{OC}$  values measured by fluorescence quenching, it should be noted that fluorescence quenching is incapable of distinguishing PAH bound to DOM from PAH bound to sediments. If the Sep-Pak separation really is capable of distinguishing freely-dissolved anthracene from anthracene bound to DOM, then one can calculate equilibrium partition coefficients omitting and including the fraction bound to DOM. At a solids-to-solution ratio of 500 mg/L, the average measured  $K_{OC}$  value ignoring DOM for radiolabelled anthracene binding to OR sediment was  $1.9 \times 10^4$  mL/g.

Table 13. Distribution of Radiolabelled Anthracene Among Various Phases in an OR Sediment Binding Experiment at Two Different Sediment Concentrations. The Mass of Anthracene Associated with Each Phase is Expressed in Counts per Minute (cpm).

-----			
	[sediment] = 100 mg/L		
	Anthracene (cpm)		Average % in Each Fraction
	Rep I	Rep II	
Total	4436	4370	-----
Freely-Dissolved	3772	3849	86.55
Sediment Bound	335	233	6.44
DOM Bound	329	288	7.01
-----			
	[sediment] = 500 mg/L		
	Anthracene (cpm)		Average % in Each Fraction
	Rep I	Rep II	
Total	4617	4469	-----
Freely-Dissolved	3486	3419	76.00
Sediment Bound	859	794	18.18
DOM Bound	272	256	5.82
-----			

This compares to  $3.6 \times 10^4$  determined by fluorescence quenching. If the amount of anthracene bound to DOM is included in the total amount bound (as the fluorescence quenching method does), the calculated  $K_{oc}$  value is  $2.5 \times 10^4$  mL/g.

At a solids concentration of 100 mg/L, the average calculated  $K_{oc}$  value ignoring DOM for radiolabelled anthracene was  $2.3 \times 10^4$  mL/g. If the amount of anthracene bound to DOM is included, the  $K_{oc}$  value jumps to  $5.2 \times 10^4$  mL/g reflecting the dependence of  $K_{oc}$  on solids concentration as well as the previously experienced dependence of PAH-DOM  $K_{oc}$  on DOM concentration when using the Sep-Pak procedure.

This data supports the conclusions of many researchers who have reported the dependence of  $K_{oc}$  on the concentration of solids in the system; however the primary purpose of these experiments was not to investigate this phenomenon. The important result here is the apparent independence of  $K_{oc}$  on solids concentration using the fluorescence quenching technique. During the course of these fluorescence quenching titrations, the concentration of sediment in the system increased from 0 to 270 mg/L. If  $K_{oc}$  was really decreasing as a function of increasing sediment concentration then this would be reflected in a nonlinear Stern-Volmer plot with a decreasing slope at higher sediment concentrations. Nonlinear plots were not found.

The reason that  $K_{OC}$  values determined by fluorescence quenching are not subject to a solids concentration effect is most likely due to the fact that fluorescence quenching accurately measures the amount of free PAH in solution. Thus, organic matter that leaves the surface of the sediment and binds free PAH in solution is accounted for. Other methods measure the amount of free pollutant but are incapable of distinguishing freely dissolved pollutant from pollutant bound to DOM. As a result, the concentration of freely dissolved pollutant is overestimated and consequently the binding constant is underestimated.

## CHAPTER 4

### CONCENTRATION OF PAH IN GREAT BAY SEDIMENTS

#### Introduction

Throughout the course of this study a number of humic materials were investigated for their PAH binding affinity. Several humic acids were derived from surficial sediments sampled from Great Bay estuary near Portsmouth, NH. Since the organic matter content of sediments is the primary controlling factor in the binding of hydrophobic organic pollutants, it was of interest to determine PAH concentrations in Great Bay sediments and to correlate these concentrations with the quality and quantity of organic matter associated with those sediments.

Sediments from six different locations (Stations 1-6, Figure 16) were examined in this study. Each sediment was analyzed for its organic matter content. In addition, four of the sediments, (Stations 1,2,4, and 6) were extracted with base to remove humic acids which were used in pyrene association experiments. It was anticipated that variations in humic acid affinity for pyrene might reflect PAH concentrations in the sediments, although sediment PAH concentrations also depend on sedimentation rate, depth of sediment sampled, particle size, and organic matter content (Pavlou and Dexter, 1979).

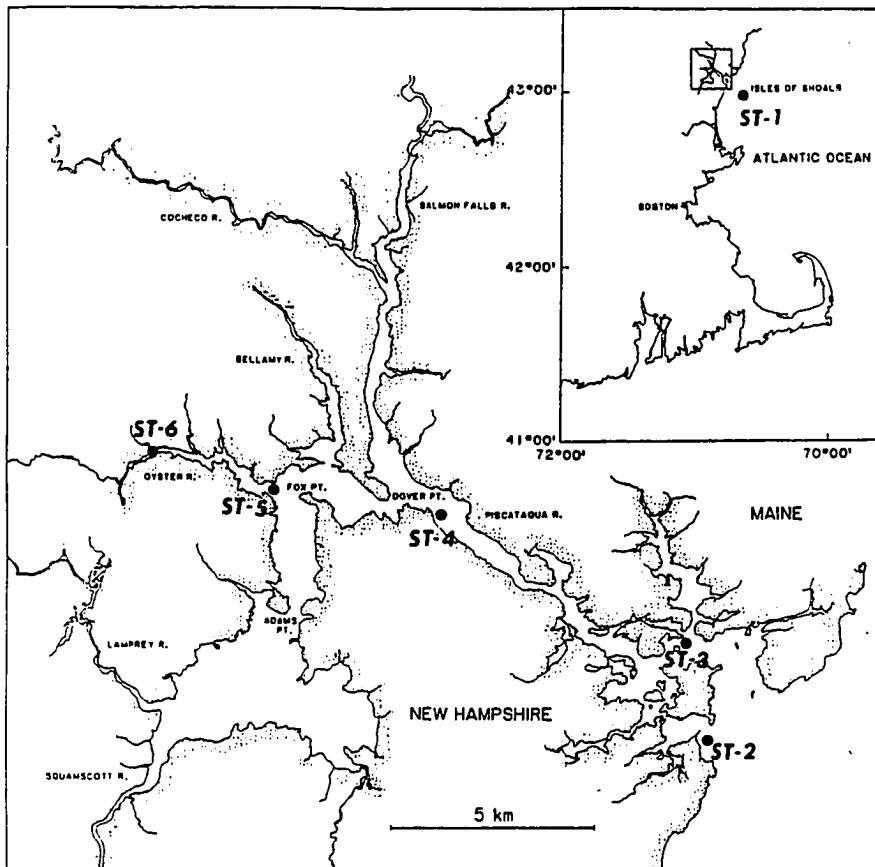


Figure 16. Map of Great Bay Estuary Depicting the Locations of Sediment Sampling Stations.

## Experimental

**Materials.** Benzene (Baker 'Resi analyzed'), methanol (E.M. Science 'Omni Solv'), and pentane (Baker 'Resi analyzed') were obtained from VWR Scientific and used without further purification. Methylene chloride (Reagent Grade) was obtained from VWR and freshly distilled over calcium hydride before use.

Sephadex LH-20 was obtained from Pharmacia Fine Chemicals. Silica gel (Supelcosil 100 - 200 mesh) was activated before use. Activated copper (20 - 30 mesh) was prepared by stirring in a beaker with 2M HCl, followed by washing with water, then methanol, and finally air drying.

PAH reference compounds (Aldrich, Sigma, Kodak, and Baker) were used as received from the suppliers.

The estuarine and ocean sediment samples were collected with a grab sampler at six sampling stations (Figure 16). Only the surface layer (0-2 cm) of recent sediments was sampled. All sediments were stored on ice while on board ship and then freeze dried after returning to the lab. The organic matter content of the sediments was determined by William Guerin of the UNH microbiology department by first drying overnight at 95°C to remove water and then by weight loss on ignition at 575 °C for 24 hours. Loss on ignition likely overestimates organic carbon but it should reveal valid trends in carbon content.

**Extraction and Isolation of PAH.** The extraction

procedure was generally as described by Giger and Schaffner (1978). A known weight of freeze dried sediment was placed in a pre-extracted glass extraction thimble fitted with a glass frit. Next, 100 uL of the extraction standard, 3,6 dimethylphenanthrene (DMP), was added to the sample. Preliminary extractions revealed no measureable amounts of DMP in the samples. The thimble and contents were placed in a soxhlet apparatus and extracted with 120 mL of methylene chloride for 6 hours. The extract was then placed in a rotary evaporator (Buchi Rotovapor-R) and concentrated under slight vacuum at 30°C to a volume of 1-2 mL. Elemental sulfur was removed by passing the extract through a column containing activated copper. The extract and several washings of methylene chloride were collected and evaporated to dryness on the rotary evaporator. Dried extract was taken up in 3-5 mL of benzene/methanol (1:1) and applied to a 50 x 2.5 cm column containing 25.0 g of Sephadex LH-20. Elution was achieved with a total of 170 mL of benzene/methanol. The first 70 mL were discarded before 100 mL were collected back in the flask. The cutoff points were determined by adding a mixture of naphthalene and pyrene to the column and determining when they eluted. Naphthalene was visually detected with UV radiation. Pyrene was detected by collecting fractions and analyzing them with a spectrofluorometer.

The second fraction was evaporated to dryness, taken up in 1-2 mL of pentane, and applied to a 1 cm i.d. column



containing silica gel (10 mL bed volume). The sample was eluted with 20 mL of pentane (discarded) followed by 25 mL of methylene chloride. This fraction was collected in a micro Kuderna Danish fitted with a condenser, and concentrated to a known volume. Finally, 100  $\mu$ L of the injection standard, hexamethylbenzene was added to the sample which was then transferred by syringe to a glass vial fitted with a Teflon lined septum and stored at 0°C in the dark.

**Gas Chromatography.** Gas chromatography was performed on a Shimadzu GC-9A equipped with a 15m x 0.25 mm i.d. SPB-1 fused silica column and flame ionization detector. Samples dissolved in methylene chloride were injected in a splitless mode with the injection port temperature held at 280 °C and the initial column temperature set at 60 °C. After 4 minutes, the split valve was opened allowing the septum and injection port to be purged at a flow rate of 80 mL/min. After elution of the solvent, the column temperature was ramped to 120 °C at 10 °C/min. and then ramped to 260 °C at 5 °C/min. and held at 260 °C for 25 minutes. Prepurified nitrogen was used as the carrier gas.

Individual PAHs were quantified using corresponding external standards and DMP as an extraction standard. Hexamethylbenzene (HMB) was intended to be used as an injection standard; however, the concentration of stock HMB was found to change over time due to an improperly sealed

vial. Injection efficiencies began at 98% and rose to 327% reflecting the evaporation of solvent and increasing concentration of HMB injected. As a result, a 100% injection efficiency was assumed for quantitation purposes.

### Results and Discussion

In general, PAH mixtures extracted from the six sediment samples were of similar composition. The gas chromatogram of the PAH mixture extracted from Station #1 sediment (Figure 17) is typical of chromatograms obtained in this study. Three and four ring PAHs were easily separated and quantified. Those PAHs quantified include naphthalene = NAP, fluorene = FL, phenanthrene = PHEN, anthracene = ANTH, 2 methyl phenanthrene = 2MP, fluoranthrene = FLUOR, pyrene = PYR, chrysene = CHR, benzanthracene = BENZ, and benzo [a] pyrene = BaP. A large number of compounds other than PAHs also appear in the chromatograms but they do not interfere with the quantitation of any PAH studied except for methyl phenanthrenes. Others have identified these compounds as large polyunsaturated aliphatic hydrocarbons (Ramos and Prohaska, 1981).

Table 14 contains results of PAH determinations for each of the sediments studied. Concentrations of PAH are expressed in ug/g of dry weight sediment (ppm). The value representing total PAH concentration is the sum of all PAHs

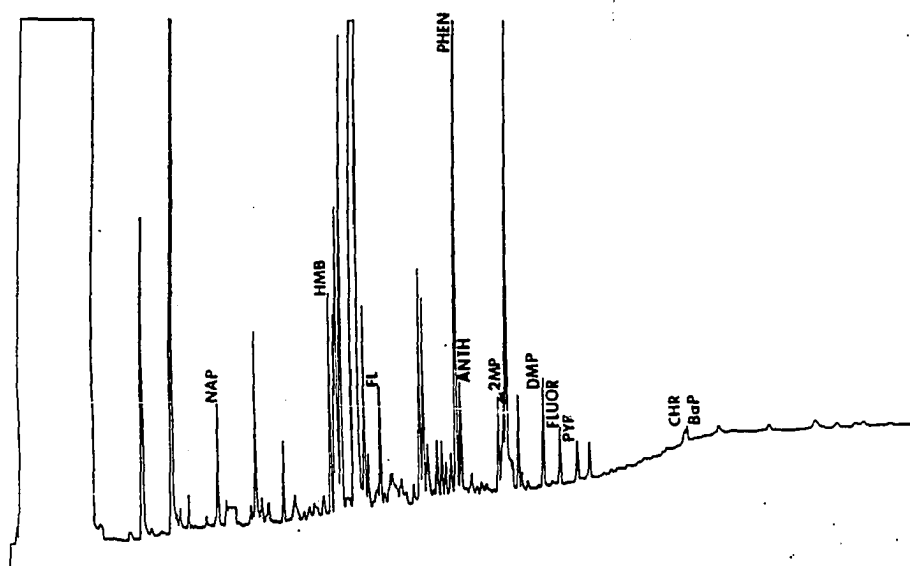


Figure 17. Capillary Gas Chromatogram of PAH Extract from Station-1 Sediment.

Table 14. Concentrations of Individual and Total PAH (ppm), % Loss on Ignition, and 2MP/PHEN Ratio Found in Sediments from Stations 1-6.

SAMPLING STATIONS	CONCENTRATION OF PAH (ppm)					
	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6
NAP	0.040	0.065	0.045	0.015	0.040	0.020
FL	0.050	0.103	0.051	0.078	0.081	0.143
PHEN	0.430	1.380	0.539	0.863	0.471	0.766
ANTH	0.067	0.384	0.103	0.118	0.492	0.147
2MP	0.091	0.240	0.122	-----	0.129	0.202
FLUOR	0.220	2.130	1.040	1.150	0.758	1.500
PYR	0.270	1.980	1.380	1.660	1.260	2.120
CHR	0.197	2.130	1.150	1.500	1.520	1.950
BENZ	0.288	2.160	1.180	1.290	1.140	2.000
BaP	0.194	1.600	0.845	0.677	0.768	0.693
TOTAL	1.76	11.93	6.33	7.35	6.53	9.34
% Loss on Ignition	2.63	10.59	7.28	3.10	7.38	8.65
2MP/PHEN	0.21	0.17	0.22	----	0.27	0.26

NAP = naphthalene, FL = fluorene, PHEN = phenanthrene, ANTH = anthracen, FLUOR = fluoranthene, PYR = pyrene, CHR = chrysene, BENZ = benzanthracene, and BaP = benzo[a]pyrene.

quantified except for 2MP. Extraction efficiencies for each of the sediments were similar and ranged from 31% to 44% except for the extraction efficiency of Station #4 sediment which was 80%. A possible reason for this high extraction efficiency will be discussed later.

In the past, the ratio of methyl phenanthrenes to phenanthrenes MP/PHEN has been used as an indicator of the relative levels of fossil and combustion derived phenanthrenes in sediment PAH mixtures (Prahl and Carpenter, 1983). PAH mixtures found in unburned fossil fuels such as petroleum and oil shales possess a greater relative abundance of alkylated PAH compared to corresponding nonalkylated PAH. Values of MP/PHEN range from 2-6 in petroleum whereas typical values for combustion PAH mixtures range from 0.5 to 1.0. The MP/PHEN value for the PAH mixtures analyzed here were all very similar and ranged from 0.17 to 0.27 indicating a combustion origin. The values are somewhat lower than typical values since only the 2 methyl isomer was quantified.

Based upon the total PAH quantified, sediment from station #2 has the highest concentration of PAH followed by sediment from stations # 6, 4, 5, 3, and 1. Referring to the map in Figure 16 there does not appear to be any relationship between the concentration of PAH in the sediment and the location of the sampling stations relative to the ocean. However, there is a very good relationship between the total PAH quantified and the organic matter

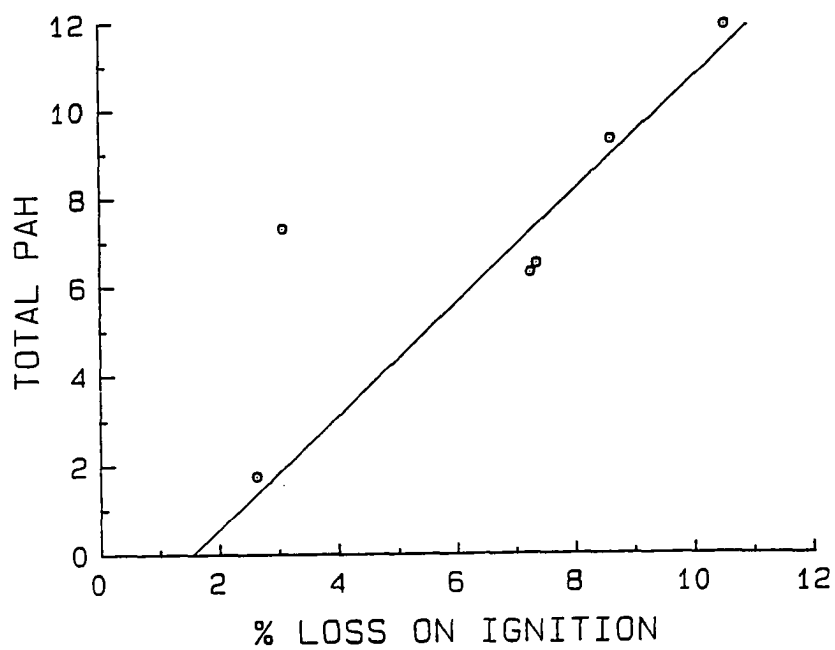


Figure 18. Least Squares Relationship Between the % Loss on Ignition and the Concentration of Total PAH Analysed in Great Bay Sediments.

content of the sediments as revealed by loss on ignition (Figure 18).

Boehm and Farrington (1984) also found a strong positive correlation between the concentration of large PAH molecules and the total organic carbon (TOC) in the sediments. The high levels of TOC were interpreted as resulting from areas of high sedimentation rates and thus a strong relationship between the depositional environment and PAH content was inferred. Prah1 and Carpenter (1983) came to a similar conclusion after investigating PAH phase associations in Washington coastal sediments. They found PAHs to be concentrated in the low density fraction of sediment which contained the highest percentage of carbon. However, they reported that humic materials extracted from sediments and subsequently analyzed for PAHs contained at most only 1% of the level of any individual PAH found in the bulk sediment.

The sediment from station #4 does not fit the correlation very well. Curiously, this sediment had an extraction efficiency of twice the average value reported for the other 5 sediments. The high extraction efficiency could be a consequence of an abnormally high PAH concentration in the sediment relative to its natural organic content. Fowlie and Bulman (1986) reported that extraction efficiencies of  $^{14}\text{C}$  labeled PAHs from contaminated soils were greater at a 50 ug/g contamination level than at 5 ug/g. Jenkins and Grant (1986) extracted

TNT from two soils where the level of contamination differed by a factor of 100 and found slower kinetics of TNT desorption for the less contaminated soil. They hypothesized that at the lower level of contamination, TNT is preferentially adsorbed to high energy binding sites. Interestingly station #4 is located near the Sprague oil refinery and thus may be subject to an increased PAH burden which may result in a temporary elevation of PAH concentration in the sediments. Others have used a point source explanation to account for similar anomolous behavior (Boehm and Farrington, 1984).

In view of the very good correlation between amount of organic matter and total PAH, it appears that the distribution of PAH in Great Bay sediments is governed primarily by the organic matter content of the sediments. This result would be expected if the organic matter associated with each of the sediments was compositionally similar. In fact, the humic acids extracted from sediments sampled at stations # 1, 2, 4, and 6 (MH-1, MH-2, MH-4, and MH-6) were very similar in nature and equally effective in sorbing pyrene. Their carbon, hydrogen, and nitrogen analyses and ratios of H/C and C/N reflect a common origin as required by this hypothesis. Since the organic content of sediments varies with the currents present at different locations, i.e. scoured areas compared to quiescent coves, such physical processes are apparently a major factor in determining distribution. In a similar situation, the major



factor reported for determining the distribution of contaminants in sediments of the Hudson-Raritan estuary was the accumulation pattern of recent fine-grained particles (Olsen and Parker, 1985).

## CHAPTER 5

### SUMMARY AND CONCLUSIONS

As a class of pollutants, PAHs deserve our attention because of their ubiquitous distribution and exceptional carcinogenicity. Because PAHs tend to associate with sediments, suspended solids and DOM in natural waters, knowledge of the amount of PAH associated with these various phases is required before toxicological impact can be accurately assessed. However, measuring the association of PAH with DOM is a difficult task due to the complex nature of DOM. Methods requiring separation of free from bound pollutant often yield suspect estimates due to the uncertainties in accomplishing a complete separation.

The results of this study show that for compounds with high fluorescence efficiencies, notably PAHs, the fluorescence quenching technique offers significant advantages. The major advantage is the absence of a separation step, thus eliminating possible errors due to incomplete separations or disruption of equilibria. Fluorescence is inherently sensitive enabling measurements of very low PAH concentrations. Furthermore, the quenching technique involves a ratio measurement so that it is not necessary to know the exact PAH concentration. This is especially useful since PAHs are only very slightly soluble in aqueous solution making it difficult to prepare solutions

of accurately known concentrations. The technique is relatively rapid (complete analysis in less than one hour) and offers very good precision (3.8 % RSD).

Fluorescence quenching was used to investigate the effects of pH, ionic strength, and humic acid composition on measured pyrene-humic acid  $K_{oc}$  values. Variations in pH from 5 to 8 did not produce a significant effect. However, the ionic strength of the solution affected the measured  $K_{oc}$  in a complex manner. At a low initial ionic strength, small additions of sodium chloride caused a relatively sharp decrease in  $K_{oc}$  values. Further additions of sodium chloride beyond an ionic strength of 0.1M caused  $K_{oc}$  values to slowly increase, approaching initial values.

It is believed that two separate effects are responsible for producing this behavior: the Fuoss effect and the ionic strength effect. As the ionic strength increases, the humic acid polymer contracts and decreases in size due to neutralization of charged functional groups. At the same time, as the ionic strength increases, the aqueous solubility of pyrene decreases. At low initial ionic strengths the Fuoss effect dominates and pyrene sorption is decreased due to a decrease in size of the humic acid. At ionic strengths beyond approximately 0.1M, it is believed that maximum contraction of the humic acid diminishes the Fuoss effect and salting out effects dominate. The aqueous solubility of pyrene decreases and  $K_{oc}$  increases.

Overall,  $K_{oc}$  values varied by as much as a factor of 3 depending upon the ionic strength of the solution, implying that the ionic strength of the solution should be carefully controlled when estimating  $K_{oc}$  values in the laboratory. Furthermore, any model designed to predict transport in an estuary where salinity can vary widely must incorporate this variable.

The most important variable affecting pyrene  $K_{oc}$  values was the elemental composition and functional group character of the humic material. Humic acid extracted from sediments and soils of different geographic origin varied by as much as an order of magnitude in their ability to sorb pyrene. Carbon, hydrogen, and nitrogen analysis along with UV, infrared and  $^{13}C$  solid state NMR spectroscopy were used to investigate compositional and structural variations in humic materials. Three different measures of the degree of C=C bond formation (UV absorptivity at 272 nm, H/C ratio, and fraction of aromatic carbon determined by  $^{13}C$  NMR spectroscopy) indicate that pyrene is preferentially sorbed by humic acids possessing a higher degree of aromaticity.

In the only other study of this nature, Diachenko (1981) reported that HCBD was preferentially sorbed by humic materials possessing a higher degree of aliphatic carbon. These two results together indicate that the binding of organic pollutants to dissolved humic materials involves, at least to some extent, specific interactions between pollutant and DOM. For the binding of PAHs to DOM the

specific interaction is believed to be pi - pi interactions between the condensed ring structure of the PAH and the condensed ring structure of portions of the humic acid molecule. The fact that binding involves specific interactions suggest that attempts to predict pollutant-DOM distribution coefficients based on solubility or octanol/water partition coefficients should include an added parameter to account for compositional and structural variations in the organic matter.

Variations in the concentration of stock humic acid solution used in the fluorescence quenching experiment also produced a change in the measured  $K_{oc}$  value. Although this variable was not examined in detail, it is believed that changes in the humic acid concentration affect the state of agglomeration of the humic material. A concentrated stock solution promoted a higher degree of agglomeration which provided a better environment for pyrene sorption. Humic acid agglomeration provides a rational explanation for anomolous DOM concentration effects reported by some authors. It may also have serious implications on the magnitude of pH and ionic strength effects which also serve to agglomerate humic materials.

Fluorescence quenching was also useful in obtaining quantitative information on the sorption of anthracene to an estuarine sediment sample. The measured  $K_{oc}$  values were similar for anthracene binding to OR sediment and anthracene binding to humic acid extracted from OR sediment implying

that the humic acid component of the sediment controls binding. However, when OR sediment was extracted with base to partially remove humic materials, anthracene binding increased by a factor of two. Since a significant amount of carbon remained associated with the sediment after extraction, it was hypothesized that the base insoluble fraction of humic material, humin, remained bound to the sediment. This material is generally considered to be highly aromatic and so it is not surprising that anthracene  $K_{oc}$  values increased. It was also observed that  $K_{oc}$  values estimated by radiolabelled PAH without separations on the aqueous phase are biased low due to failure to account for binding by DOM.

Analysis of PAH in Great Bay sediments revealed a very good correlation between the total PAH estimates and the organic content of the sediments. It was hoped that variations in the composition of the humic matter might reflect variations in bound PAH; however, Great Bay humic acids were compositionally similar and this concept could not be adequately tested. Instead, this study presents strong evidence that temporal mean concentrations of resistant organic pollutants in sediments can be adequately predicted based on the organic matter content of the sediments.

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