Interactions of hydrophobic organic solutes with dissolved humic substances
by Shaojin Chen

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Science
Montana State University

Abstract:
Interactions of hydrophobic organic solutes with dissolved humic substances may have a significant effect on the chemistry and fate of contaminants in natural systems. Fluorescence quenching, headspace gas chromatography (GC) and phytotoxicity bioassays were used to assess the complexation of nonionic, hydrophobic organic solutes with humic substances. No increase in quenching at elevated temperature, an increase in quenching ratio at higher viscosity, and no significant shortening of the fluorescence lifetimes of the solutes in the presence of the quencher indicated that the primary fluorescence quenching mechanism was static, resulting from the formation of solute-humic complexes. Fluorescence quenching studies with fluoranthene, 1-naphthol and napropamide, static headspace GC studies with herbicides dichlobenil, triallate and trifluralin showed significant complexation of these solutes with a variety of dissolved humic (HA) and fulvic acids (FA). The ionic strength of the solution adjusted using KCl did not significantly affect the complexation and Henry's law constants, but an increase in pH resulted in more complexation for 1-naphthol. Conditional complexation constants ranged from 9.7 to 91.5 L/g C for these solutes and generally increased with increasing solute hydrophobicity, suggesting a hydrophobic partitioning mechanism. The complexation of 1-naphthol with HA was enhanced in the presence of Cu^{2+} and Zn^{2+} at a pH higher than the pKa of 1-naphthol (9.34). This suggests that cation bridging between functional groups of anionic organic solutes and HA or FA is an additional binding mechanism for the association of organic solutes with soluble humic substances. Generally, HA showed stronger affinity for complexation with these hydrophobic organic solutes than FA. Bioassay studies with oats and tomatoes as indicator species showed that the phytotoxicity of atrazine, picloram and triallate was reduced in the presence of HA, probably resulting from reductions in bioavailability of these herbicides due to complex formation. In summary, a significant portion of these organic solutes may exist as water soluble complexes of dissolved humic substances in aqueous systems. The complexation of organic solutes with humic substances is an important process in determining the behavior and fate of these chemicals in natural systems.

INTERACTIONS OF HYDROPHOBIC ORGANIC SOLUTES WITH DISSOLVED HUMIC SUBSTANCES

by

Shaojin Chen

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Bozeman, Montana

April, 1992

APPROVAL

of a thesis submitted by

Shaojin Chen

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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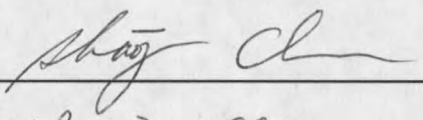
Signature 

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VITA

Shaojin (Sam) Chen was born in Raoping, Guangdong, China on October 26, 1961. He is married to Zihui Zhang.

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ABSTRACT

Interactions of hydrophobic organic solutes with dissolved humic substances may have a significant effect on the chemistry and fate of contaminants in natural systems. Fluorescence quenching, headspace gas chromatography (GC) and phytotoxicity bioassays were used to assess the complexation of nonionic, hydrophobic organic solutes with humic substances. No increase in quenching at elevated temperature, an increase in quenching ratio at higher viscosity, and no significant shortening of the fluorescence lifetimes of the solutes in the presence of the quencher indicated that the primary fluorescence quenching mechanism was static, resulting from the formation of solute-humic complexes. Fluorescence quenching studies with fluoranthene, 1-naphthol and napropamide, static headspace GC studies with herbicides dichlobenil, triallate and trifluralin showed significant complexation of these solutes with a variety of dissolved humic (HA) and fulvic acids (FA). The ionic strength of the solution adjusted using KCl did not significantly affect the complexation and Henry's law constants, but an increase in pH resulted in more complexation for 1-naphthol. Conditional complexation constants ranged from 9.7 to 91.5 L/g C for these solutes and generally increased with increasing solute hydrophobicity, suggesting a hydrophobic partitioning mechanism. The complexation of 1-naphthol with HA was enhanced in the presence of Cu^{2+} and Zn^{2+} at a pH higher than the pK_a of 1-naphthol (9.34). This suggests that cation bridging between functional groups of anionic organic solutes and HA or FA is an additional binding mechanism for the association of organic solutes with soluble humic substances. Generally, HA showed stronger affinity for complexation with these hydrophobic organic solutes than FA. Bioassay studies with oats and tomatoes as indicator species showed that the phytotoxicity of atrazine, picloram and triallate was reduced in the presence of HA, probably resulting from reductions in bioavailability of these herbicides due to complex formation. In summary, a significant portion of these organic solutes may exist as water soluble complexes of dissolved humic substances in aqueous systems. The complexation of organic solutes with humic substances is an important process in determining the behavior and fate of these chemicals in natural systems.

CHAPTER 1

INTRODUCTION

The interaction between organic solutes (pesticides and industrial chemicals) and water soluble humic substances can influence the behavior and fate of these solutes in natural systems. Solute properties including bioactivity, persistence, biodegradability, leachability and volatility have been shown to be affected by complexation with soluble humic substances (Ballard, 1971; Bollag, 1983 ; Carter and Suffet, 1983; Landrum et al., 1987; Spencer et al., 1988; West, 1984). For example, according to Ballard (1971), the downward movement of the insecticide DDT in the organic layers of forest soils is caused by binding (complexation) of DDT with water soluble humic substances. Other studies on the interactions between dissolved humic (HA) and fulvic (FA) acids and organic pollutants have shown an increase in the water solubility of these compounds in the presence of humic substances (Chiou et al., 1986). Numerous studies have shown that the adsorption, leaching and bioaccumulation of hydrophobic organic solutes are correlated with their water solubilities (Chiou et al., 1977; Hassett et al., 1981; West, 1984). Consequently, the water solubility of a compound is a key factor influencing its behavior and fate in soils and its tendency to form complexes with soluble humic substances.

Humic substances can be divided into two groups according to their original sources: aquatic and terrestrial (soil). Although these two groups differ slightly in

elemental composition, their basic chemical structure is thought to be similar (Stevenson, 1982). Soil organic matter (SOM) is a generic term used to include all organic compounds found in soils. It is well known that SOM is comprised of a diverse and dynamic group of chemicals. Traditionally, SOM is classified into 2 groups: the non-humic and the humic substances, with the latter playing an important role in the complexation of organic solutes. Humic substances (humus) in soils are operationally defined as those organic materials extractable by strong bases, and are further divided into humin, humic acid and fulvic acid according to their water solubility and resistance to precipitation by acids (Stevenson, 1982). Recently, the definition of humic substances has been clarified to mean those organic substances in water or extracted from soils that can be adsorbed by hydrophobic XAD resins (Thurman and Malcolm, 1981). Although the theories of humus formation in soils differ considerably and the origin of humus is not well understood, it is generally agreed that humic substances are complicated heterogeneous organic compounds somewhat modified by microbial activity, with diverse functional groups and various degrees of polymerization and branching. Significant heterogeneity in composition and structure exists within and among the various fractions of humic substances (Stevenson, 1982). An important characteristic of humic substances is the simultaneous presence of aromatic ring structures and the abundance of carboxylic, hydroxylic and other hydrophilic functional groups. One very important consequence of these structural features is the presence of both hydrophobic and hydrophilic sites or subunits on the same polyelectrolyte.

More recently, the importance of the interactions between hydrophobic organic

solutes and dissolved humic substances has been recognized, especially considering the fate of xenobiotics and pesticides in the environment. It is clear that complexation of organic solutes with soluble naturally occurring humic substances is an important process in both soils and aquatic systems. However, due to the large number of pesticides and industrial chemicals, and the complexity and diversity of dissolved humic substances, our current understanding of solute-humic complexation and subsequent implications for the fate of these solutes in the environment is limited. For example, the primary binding (association) of hydrophobic organic solutes with dissolved humic substances in aqueous solutions is thought to be caused by hydrophobic partitioning (Carter and Suffet, 1982, 1983; Chiou et al., 1983, 1986, 1987). But to what extent does hydrophobic partitioning of pesticides occur in soluble organic C? Which techniques are suitable for studying the interaction of solutes with soluble organic C? How do solution conditions affect the interactions of pesticides with soluble organic C? Are there other mechanisms for binding? In addition to water solubility, what are other important physical-chemical parameters for organic solutes in nature? Does binding of an organic solute by dissolved humic substances influence such parameters? What is the effect of herbicide-humic interaction on the bioavailability and phytotoxicity of the herbicide? In order to address these questions, a study was conducted to examine the interactions between several pesticides and several dissolved humic and fulvic acids, using fluorescence spectroscopy, static headspace gas chromatography and phytotoxicity bioassays.

CHAPTER 2

COMPLEXATION OF 1-NAPHTHOL BY HUMIC AND FULVIC ACIDS

Introduction

The interaction between organic solutes and humic substances may alter the fate of pollutants in soil or aquatic systems (Ballard, 1971; Caron et al., 1985; Gschwend and Wu, 1985; Poirrier et al. 1972). Significant amounts of anthropogenic organic solutes present in aquatic systems may be in the form of soluble organic complexes, as illustrated by the study on the pesticide mirex (1,1a,2,2,3,3a,4,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1H-cyclobuta(cd)pentalene) in surface waters (Yin and Hassett, 1986). Complex formation of organic solutes with water soluble organic carbon (C) may result in water solubility enhancement of hydrophobic compounds (Chiou et al., 1986, 1987), reduction of biotoxicities, and a change in rate of bioaccumulation (Dell'Agnola et al., 1981; Landrum et al., 1987).

Complexation of nonpolar organic solutes with humic substances has resulted in a decrease in their adsorption to soil surfaces (Gschwend and Wu, 1985; Caron et al., 1985) and an increase in transport rates in porous media (Enfield and Bengtsson, 1986). A number of techniques including fluorescence quenching, solubility enhancement, gel permeation (size exclusion chromatography), equilibrium dialysis, reverse-phase separation, ultrafiltration and head space gas chromatography have been used to estimate the extent of interaction of organic solutes with dissolved organic matter (DOM), and to elucidate the mechanisms of complexation (Carter and Suffet, 1982, 1983; Chin and

Weber, 1989; Gauthier et al., 1986; Hassett and Milicic, 1985; Landrum et al., 1984; Lee and Farmer, 1989; Madhun et al., 1986; McCarthy and Jimenez, 1985; Traina et al., 1989).

Despite considerable effort from various researchers, the mechanism and extent of complexation of organic solutes with humic substances are poorly understood. McCarthy and Jimenez (1985) have shown that the association of some polycyclic aromatic hydrocarbons with soluble humic substances is fully reversible, but more recent work by Lee and Farmer (1989) demonstrated that the interaction between napropamide (N,N-diethyl-2-(1-naphthalenyloxy)-propanamide) and dissolved peat humic acid was not fully reversible. To explain the effect of dissolved humic materials on the transport of hexachlorobenzene and anthracene in soil columns, West (1984) postulated that irreversible formation of humic-solute complexes occurred during transport. Clearly, further study is needed in order to understand the interaction between organic solutes and dissolved organic C in natural systems.

Hassett et al. (1981) found that the mobility of naphthol increased as the hydrophobicity of the mobile phase (solvent) increased. They concluded that factors increasing the affinity of the solute for solvent resulted in decreased sorption and increased mobility of the solute. Consequently, one would expect a significant amount of complexation of naphthol with humic and fulvic acids. 1-Naphthol is the major component of many pesticides such as the herbicide, napropamide and the insecticide, carbaryl (methylcarbamate-1-naphthalenol). It is also the main degradation metabolite of naphthalene which is on the EPA list of priority pollutants. 1-Naphthol is relatively

nonpolar, with a pKa of 9.34 (Weast, 1985) and exists as a nonionic compound at neutral pH. Because of its fused aromatic rings, 1-naphthol fluoresces strongly and shows a fair degree of hydrophobicity (octanol-water partition coefficient, K_{ow} = 700±62, Hassett et al. 1981). Considering these properties, 1-naphthol is an ideal model compound to study the interaction between organic solutes and dissolved humic materials.

The objectives of this study were to (i) determine whether quenching of 1-naphthol fluorescence by humic acid (HA) and fulvic acid (FA) is dynamic or static, (ii) obtain the complex formation equilibrium constants of a variety of HA and FA, (iii) test the effects of solution ionic strength, pH, and cation composition on the association of 1-naphthol with HA and FA, and (iv) suggest possible modes of 1-naphthol-HA association.

Materials and Methods

Preparation Of Solutions

1. 1-Naphthol stock solution

A $1x10^{-2}$ *M* solution of 1-naphthol in methanol was prepared by dissolving high purity 1-naphthol (Aldrich Chemical Co., Milwaukee, WI. Gas chromatography-mass spectrometry analysis showed the purity was essentially 100%) into spectrum grade methanol (EM Science, Cherry Hill, NJ). The solution was transferred to a glass bottle wrapped with aluminum foil and stored at 5° C. Since this naphthol stock solution contained methanol and contributed a final methanol concentration of 0.1% (v/v) to treatments that received 1-naphthol, methanol was added to treatments that received no naphthol so that the final concentration of methanol was 0.1%. Preliminary studies

showed that the presence of 0.1% (v/v) methanol did not alter the fluorescence of 1-naphthol.

2. Dissolved humic substances solutions

Five different HA or FA were used in the fluorescence experiments with 1-naphthol (Table 1). Stock solutions of these acids were prepared by dissolving the solid phase in KOH (pH ≈ 8 - 9), filtering through 0.45 μm filters, then adjusting the pH to 7.0. The total dissolved organic carbon (DOC) of the stock solutions was measured with a Dohrmann DC-80 carbon analyzer.

Fluorescence Experiments

A typical fluorescence experiment consisted of 3 treatments in triplicate: naphthol, naphthol plus HA or FA, and HA or FA alone (Fig. 1). Twenty-five-ml volumetric flasks were used as reaction vessels and at least 12 h was allowed for equilibration before fluorescence measurements. With the exception of experiments designed to vary the ionic strength, pH, and metal cations, all other experiments were performed in 0.01 *M* KCl at pH 7.0. All fluorescence measurements were made with a Spex fluorolog-2 spectrofluorometer (Spex Industries, Inc., Edison, NJ) equipped with a 150 W Xe lamp. All fluorescence intensity measurements were made with the excitation wavelength fixed at 294 nm and emission wavelength at 468 nm, with 10 scans for each measurement. The inner filter effect was corrected for each measurement by manually adjusting the cuvette position via an x-y translation stage. The position of the micrometer was recorded for the naphthol plus HA or FA treatment and used for the intensity measurement of the HA or FA blank treatment. The effective bandpass was 2.25 nm and 4.5 nm for the excitation

Table 1. Chemical properties of humic substances used in this study.[†]

Humic Substance	C	H	O	N	S	P	Ash	COOH	OH	Percentage of
	g kg^{-1}							(mmol charge/g)	(mmol charge/g)	Aromatic[§] C
IHSS[¶] Reference HA (1R106H)	541.3	49.1	353.9	50.3	6.0	4.0	15.2	ND[‡]	ND	24
Commercial HA	399.0	39.4	301.9	5.2	ND	ND	155.6	ND	ND	ND
Montana Soil HA	432.7	40.2	354.9	30.9	ND	ND	152.4	ND	ND	ND
IHSS Standard FA (1S101F)	537.5	42.9	404.8	06.8	0.50	0.01	0.82	6.0	1.2	18
Wheat Straw FA	555.0	52.0	357.0	08.0	ND	ND	2.40	5.5	0.7	30

[†] All items on a moisture-free basis

[‡] ND: not determined

[§] Percentage Aromaticity = $\frac{\text{Peak area of } ^{13}\text{C-NMR spectrum 110-160 ppm}}{\text{Total peak area of } ^{13}\text{C-NMR spectrum 0-230 ppm}}$

[¶] IHSS = International Humic Substances Society

source ?
Method of preparation
analysis ?

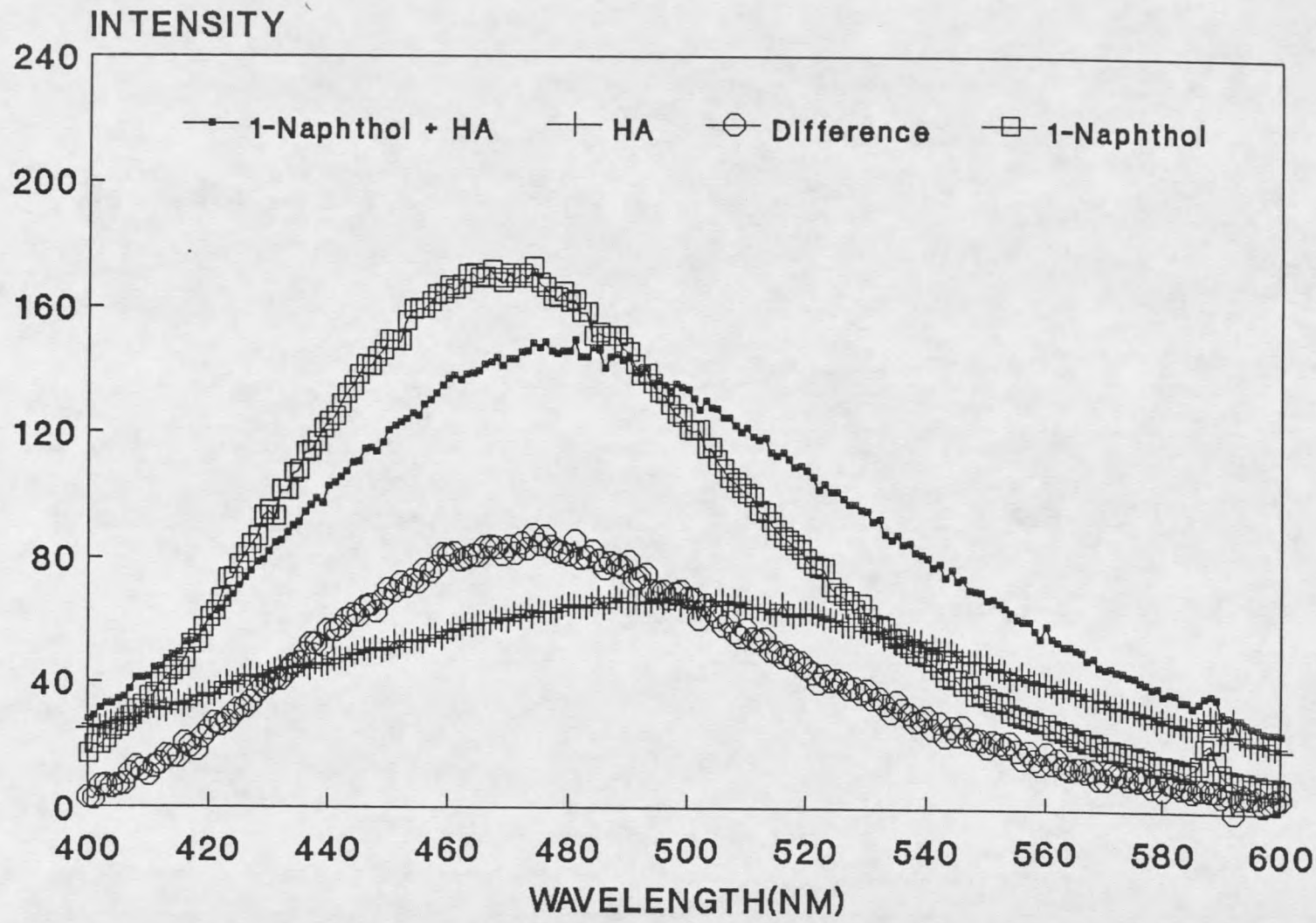


Fig. 1. Emission spectra of 1-naphthol, 1-naphthol plus HA, HA, and the difference spectrum between 1-naphthol plus HA and HA. [1-naphthol] = 1×10^{-5} *M*, commercial [HA] = 2.55×10^{-3} *M* DOC.

monochromator and the emission monochromator, respectively.

Static vs. Dynamic Quenching

Fluorescence quenching can be described by the Stern-Volmer equation (Lakowicz, 1983):

$$F_o/F = 1 + K_{sv}[Q] \quad [1]$$

where F_o and F are the fluorescence intensities in the absence and presence of the quencher Q, [Q] is the concentration of quencher, and K_{sv} is the Stern-Volmer constant. If the quenching is static (due to the formation of complexes of the fluorophore and the quencher), K_{sv} is equal to the complexation stability constant K:

$$K_{sv} = K = [F\text{-}Q]/([F][Q]) \quad [2]$$

where [F] and [F-Q] are the concentrations of free fluorophore and fluorophore-quencher complex, respectively. If the quenching is collisional (due to the collision of the excited fluorophore with the quencher molecules), the Stern-Volmer constant is the product of the bimolecular quenching constant, k_q, and the fluorescence life time, τ, of the fluorophore in the absence of the quencher:

$$K_{sv} = k_q\tau \quad [3]$$

For collisional quenching, the constant can be expressed as:

$$K_{sv} = \gamma k_0\tau \quad [4]$$

where k_0 is the bimolecular rate constant and γ is the quenching efficiency (Lakowicz, 1983).

In order to predict the influence of temperature on collisional quenching, it is assumed that $\gamma = 1$ in Eq. [4] (i.e. every collision between the excited fluorophore and the

quencher results in quenching). The Smoluchowski equation can be used to calculate k_0:

$$k_0 = 4\pi RDN/1000 = (4\pi N/1000)(R_f + R_q)(D_f + D_q) \quad [5]$$

where R is the collision radius, D is the sum of the diffusion coefficients of the fluorophore (D_f) and the quencher (D_q), and N is Avogadro's number. The collision radius is generally assumed as the sum of the molecular radii of the fluorophore (R_f) and quencher (R_q). Assuming that the collision radius does not change with temperature, and allowing $y = (4\pi N/1000)(R_f + R_q)$, we have

$$k_0 = y(D_f + D_q) \quad [6]$$

The diffusion coefficients for species i may be obtained from the Stokes-Einstein equation:

$$D_i = kT/6\pi\eta R_i \quad [7]$$

where k is the Boltzmann constant, T is the temperature, η is the solvent viscosity and R is the radius of species i. Letting $x_i = k/6\pi R_i$, Eq. [7] becomes

$$D_i = x_i T/\eta \quad [8]$$

The Stern-Volmer constants at T_1 and T_2 can be calculated from Eq.'s [4], [6] and [8]:

$$K_{sv,1} = k_{0,1}\tau_1 = y(x_f + x_q)(T_1/\eta_1)\tau_1 \quad [9]$$

$$K_{sv,2} = k_{0,2}\tau_2 = y(x_f + x_q)(T_2/\eta_2)\tau_2 \quad [10]$$

where η_1 and η_2 are the solvent viscosities at T_1 and T_2, respectively. By definition, the quantum yield $Q = \tau/\tau_o$, where τ is the lifetime of fluorescence and τ_o is the intrinsic lifetime (the lifetime of the fluorophore in the absence of nonradiative process (Lakowicz, 1983)) which is independent of T. If the conditions for the fluorescence intensity measurements are identical at temperature T_1 and T_2 (i.e. 313.15 and 283.15

K), then:

$$Q_1/Q_2 = (\tau_1/\tau_o)/(\tau_2/\tau_o) = I_1/I_2 \quad [11]$$

where Q_1 and Q_2 are the quantum yields, and I_1 and I_2 are intensities at T_1 and T_2. In the current study at $T_1 = 313.15$ and $T_2 = 283.15$ K, $I_1/I_2 \approx 0.903$ so $\tau_1/\tau_2 \approx 0.903$. For water at $T_1 = 313.15$ K, $\eta_1 = 0.6529$ cp and at $T_2 = 283.15$ K, $\eta_2 = 1.307$ cp (Weast, 1985). The ratio of Stern-Volmer constants at T_1 and T_2 can be estimated from Eq.'s [9] and [10] as

$$K_{sv,1}/K_{sv,2} = (k_{0,1}\tau_1)/(k_{0,2}\tau_2) = (T_1\tau_1\eta_2)/(T_2\tau_2\eta_1) \approx 1.999 \quad [12]$$

If the quencher concentration is constant at T_1 and T_2, then

$$\{(F_o/F)_1 - 1\}/K_{sv,1} = \{(F_o/F)_2 - 1\}/K_{sv,2} \quad [13]$$

Combination of Eq.'s [12] and [13] yields

$$(F_o/F)_{313.2\,K} \approx 1.999(F_o/F)_{283.2\,K} - 0.999 \quad [14]$$

In the presence of a quencher, $(F_o/F)_{283.2\ K} > 1$; consequently, for collisional quenching we would expect that the quenching ratio increases with temperature.

Two experiments were conducted to test whether fluorescence quenching of 1-naphthol by HA was collisional (dynamic) or static (complex-forming). In the first experiment, a brass water-jacketed cuvette holder was used to make fluorescence measurements at temperatures ranging from 283 K to 313 K($\pm 1^0$). In the second experiment, glycerol was added to the reaction vessels at 0%, 10% and 50% (v/v) to change the viscosity of the solution. Glycerol was added to all 3 treatments: 1-naphthol, 1-naphthol plus HA, and HA alone.

Effects of Solution Composition

To describe the quenching of 1-naphthol fluorescence by HA and FA, different amounts of HA or FA were added to $1x10^{-5}$ *M* 1-naphthol in 0.01 *M* KCl at pH 7. The fluorescence intensity of $1x10^{-5}$ *M* 1-naphthol in 0.01 *M* KCl at pH 7 was taken as F_o. The presence of HA or FA contributed a small amount of fluorescence to the naphthol plus HA or FA treatment, so F was determined from the difference spectra of HA or FA plus naphthol and the HA or FA blanks (Fig. 1). Linear regression was used to obtain a relationship between the quenching ratio F_o/F and the concentration of HA or FA expressed as molarity of DOC.

Two salts, KCl and $CaCl_2$, were employed to test the effects of ionic strength on fluorescence quenching. The concentration of 1-naphthol was fixed at $1x10^{-5}$ *M*, HA was fixed at $2x10^{-3}$ *M* DOC and ionic strength varied from 0.001 to 0.5 *M*. After equilibrating the reaction vessels for 12 h, we observed that concentrations equal to or greater than 0.033 *M* $CaCl_2$ caused the HA to flocculate. For these treatments, fluorescence intensity was determined only on the supernatants.

In the pH experiment, the concentration of HA was fixed at $2x10^{-3}$ *M* DOC for all the naphthol plus HA, and HA blank treatments. The desired pH values (3.0, 7.0 and 10.0) were achieved by drop-wise addition of HCl or KOH solutions.

To assess the role of cations on the formation of 1-naphthol-HA complexes, Cu and Zn were added to reaction vessels containing 1-naphthol and HA. The experiment was performed at two pH values, 5.0 and 9.5, with and without metal ions. Additions of Cu^{2+} and Zn^{2+} to the reaction vessels were made from 1 m*M* stock solutions of each ion,

which were prepared from $CuCl_2 \cdot 2H_2O$ (>99% purity, Aldrich) and $ZnSO_4 \cdot 7H_2O$(99% purity, Aldrich) respectively so that the final concentrations of Cu and Zn were $1x10^{-4}$ *M*. Several preliminary experiments were conducted to determine acceptable levels of Cu and Zn that could be used at pH 9.5 without precipitation of the metal hydroxides. At the concentrations of Cu and Zn used in these experiments, no visible hydroxide precipitates were observed.

Results and Discussion

Static vs. Dynamic Quenching

1. Temperature

Results of the temperature experiment at pH 7 show that the quenching ratio did not change significantly from 283.2 K to 313.2 K (Fig. 2). The slope of the plot F_o/F vs. temperature was essentially zero (t-test indicated that it was not significantly different than 0 at $\alpha = 0.01$). If the quenching was solely a result of collisional processes, we estimated that $(F_o/F)_{313\ K}$ would be approximately 3.74 (Eq. [14]). However, the measured quenching ratio at 313 K was 2.37, much smaller than expected. Therefore, quenching of 1-naphthol fluorescence by HA did not follow the temperature dependence expected for a collisional mechanism.

One form of the van't Hoff equation,

$$\ln(K_2/K_1) = (\Delta H^0/R)(1/T_1 - 1/T_2) \qquad [15]$$

describes the influence of temperature on the equilibrium constants, K_1 and K_2 at temperatures, T_1 and T_2 respectively, where ΔH^0 is the standard enthalpy change and R

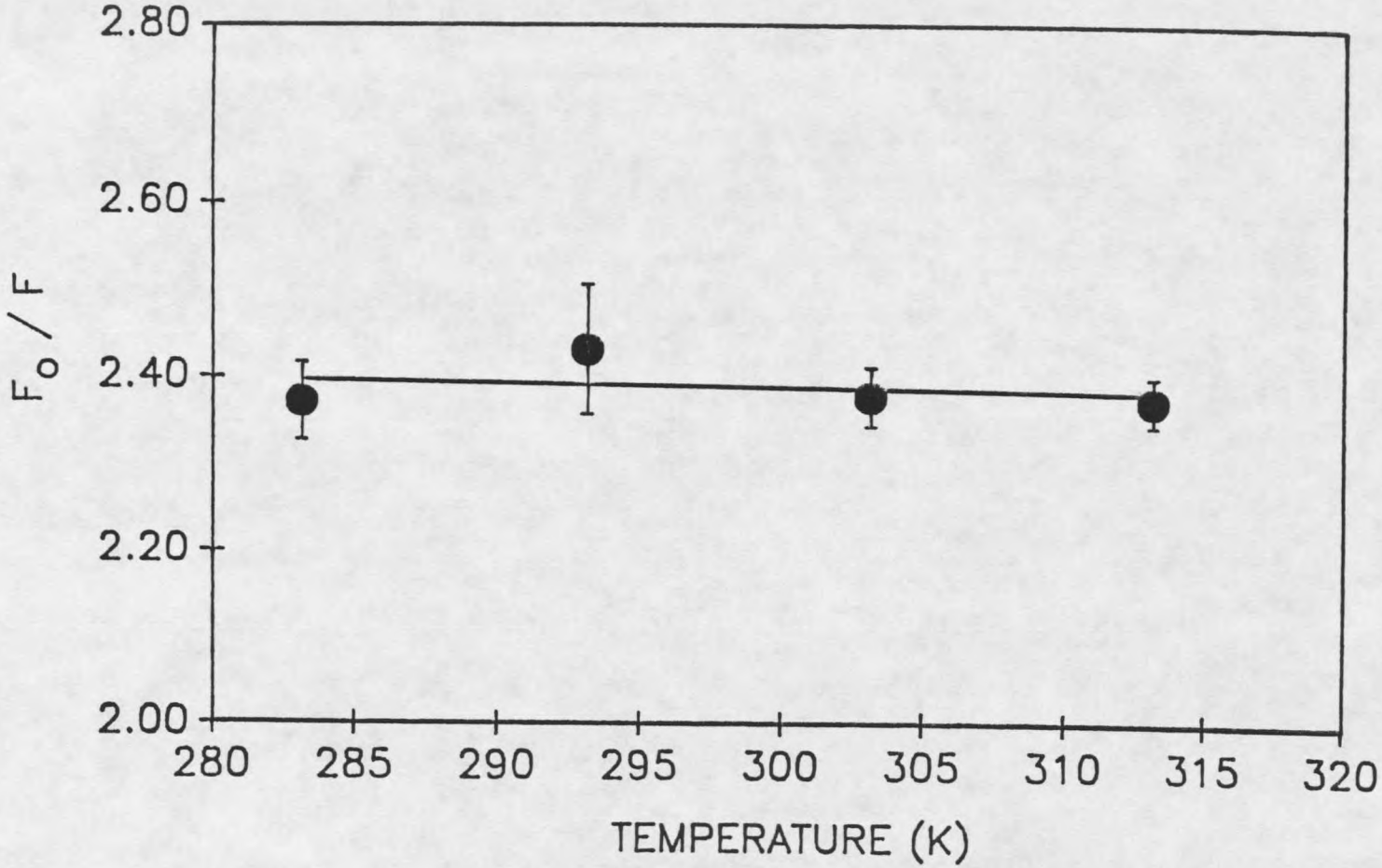


Fig. 2. Effects of temperature on the quenching ratio. [1-naphthol] = $1x10^{-6}$ *M*, IHSS [HA] = $2.00x10^{-3}$ *M* DOC.

is the gas constant. Although the equilibrium constants obtained in this study are conditional, thermodynamic parameters obtained through utilization of these constants can provide meaningful information about the complexation reaction. Since the concentration of HA was constant and the quenching ratio did not change with temperature, K_1 must equal K_2 (Eq. [13]), and $\ln (K_2/K_1) = 0$. It follows that ΔH^0 from Eq. [15] must be essentially zero, suggesting that the complexation was an entropy-driven process. This postulation, supported by our data is in agreement with results of investigations performed on compounds similar to 1-naphthol (Gauthier et al., 1987; Traina et al., 1989).

2. Viscosity

If the quenching of 1-naphthol fluorescence by HA was due to diffusion controlled collisions between the fluorophore and the quencher, one would expect that as the viscosity of the solvent increased, the rate of diffusion should decrease and the quenching ratio (F_o/F) should also decrease. When the solvent viscosity was raised by increasing the concentration of glycerol to 10 and to 50%, the quenching ratio F_o/F increased considerably compared to 0% glycerol (Table 2). This is opposite to the trend expected fluorescence was due to collisional processes. Although the introduction of glycerol to the system may alter the chemical environment of the fluorophore and the HA, all treatments (naphthol alone, naphthol plus HA, and HA alone) received glycerol so that the comparison was made on a relative basis. Two reasons may explain why F_o/F increased with increasing glycerol concentration: an increase in apparent HA concentration due to the water-absorbing property of glycerol and a higher level of

Table 2. Effects of solution viscosity (glycerol concentration) on the quenching ratio, F_o/F ([1-naphthol] = 1 x 10^{-5} *M*, commercial humic acid = 1.3 m*M* DOC).

Glycerol Concentration (v/v%)	F_o/F
0	1.48±0.05
10	1.69±0.09
50	2.40±0.23

± Values are standard deviations of three replications.

exposure of hydrophobic regions of HA due to formation of H-bonds between the hydrophilic hydroxylic and carboxylic groups of glycerol and HA.

3. Bimolecular Quenching Rate

Further insight into the mechanism of quenching of 1-naphthol fluorescence by HA can be obtained by examining the bimolecular quenching constant, k_q, assuming a dynamic quenching mechanism. From Eq. [3], $k_q = K_{sv}/\tau$. The Stern-Volmer constant for the International Humic Substance Society (IHSS) HA was 241 L/mol C or 10.9 L/gm HA (Fig. 3). The molecular weight (M.W.) for humic acids can range from 2600 to 200000 (Stevenson, 1982). In a case where the M.W. = 50000, $K_{sv} = 5.4 \times 10^5$ L/mol HA. The fluorescence lifetime, τ of 1-naphthol in an organic solvent was found to equal 10.6×10^{-9} s (Becker, 1969). Consequently, $k_q = 5.1 \times 10^{13}$ $M^{-1}s^{-1}$. Similarly, if the M.W. = 2600,

then $k_q = 2.7 \times 10^{12}\ M^{-1}s^{-1}$. A k_q near $1 \times 10^{10}\ M^{-1}\ s^{-1}$ is considered an upper limit for diffusion processes in aqueous solution (Lakowicz, 1983). Consequently, the Stern-Volmer constants appear to reflect a static quenching mechanism. Other studies in aqueous solutions at pH 7 have shown that the main species emitting fluorescence was the naphthol anion due to the low pK_a for the excited state naphthol. The fluorescence lifetimes for the anion and neutral molecule were measured as $8.0 \pm 0.2 \times 10^{-9}$ and $8.0 \pm 0.4 \times 10^{-9}$ s, $0.8 \pm 0.4 \times 10^{-9}$ and $1.50 \pm 0.3 \times 10^{-9}$ s (Harris and Selinger, 1980; Webb et al., 1986). Using these lifetimes results in only larger values of k_q.

Stern-Volmer Constants

A typical Stern-Volmer plot of 1-naphthol fluorescence quenched by HA and FA is shown in Fig. 3. Table 3 summarizes the plots and the association (binding) constants calculated on the basis of DOC. All the linear regression equations show excellent fit to the Stern-Volmer equation, and the extent of quenching by FA was less than by HA as evidenced by the smaller K_{sv} values. This can be explained in terms of a hydrophobic association considering that HA generally contains more hydrophobic regions than FA. A comparison of K_{oc} (partition coefficient normalized to organic C) values for several polycyclic aromatic hydrocarbons binding with dissolved humic substances revealed that the extent of complexation increases with the hydrophobicity of the solute (Table 4).

Effects Of Solution Parameters

1. Ionic Strength

An increase in the ionic strength (I) of the solution from 0.001 *M* to 0.5 *M* did not significantly change the quenching ratio or the complexation reaction with KCl as the salt

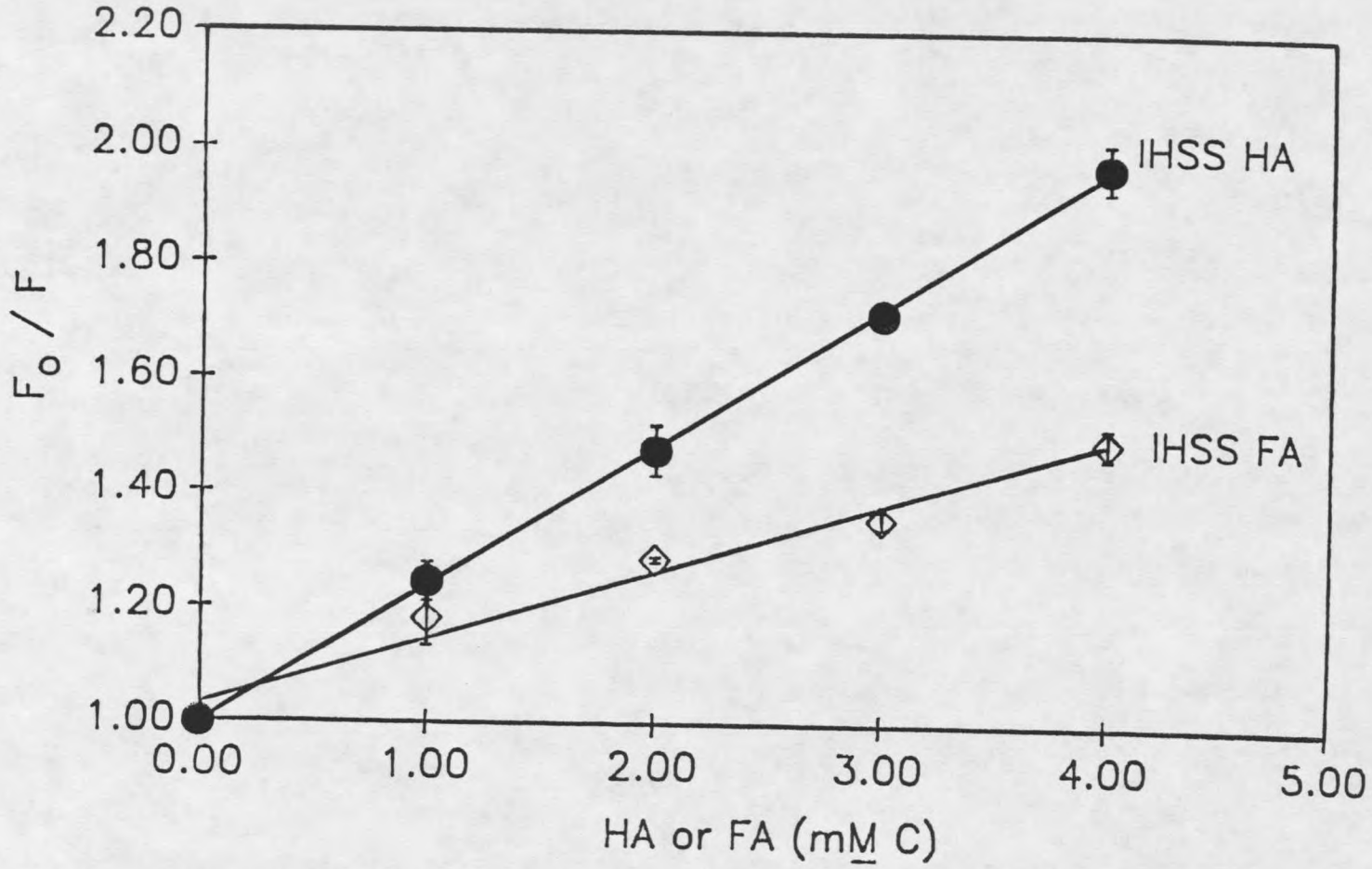


Fig. 3. Stern-Volmer plots of 1-naphthol fluorescence quenched by IHSS reference humic acid and standard fulvic acid. [1-naphthol] = $1x10^{-5}$ *M*.

Table 3. (F_o/F) vs concentration of quencher (m*M* DOC) to the Stern-Volmer equation (Eq. 1) and calculated K_{oc} values for humic and fulvic acids used in the current study.

Quencher	Linear Correlation R^2	K_{oc} (L/g C)
IHSS HA	0.998	20.1
Commercial HA	0.998	31.4
Montana Soil HA	0.998	33.4
IHSS Standard FA	0.979	9.7
Wheat Straw FA	0.999	10.0

(Fig. 4). Identical results were obtained with $CaCl_2$ at ionic strengths < 0.01 *M*; however, when ionic strength reached 0.100 *M*(0.033 *M* $CaCl_2$), HA flocculents were visible and the quenching ratio dropped considerably (Fig. 4). Calcium binds relatively strongly with carboxylic functional groups of HA, causing the polymer to precipitate. The fact that F_o/F decreased from 1.5 to 1.2 when $CaCl_2$ exceeded 0.033 *M* indicates that HA-naphthol complexes dissociated upon HA flocculation, and a portion of the naphthol either remained bound to the flocculants or to soluble organics left in solution. The probable mechanism of naphthol complexation with HA at pH = 7.0 is an entropy driven

Table 4. Comparison of K_{oc} values determined from fluorescence quenching experiments on a variety of polycyclic aromatic hydrocarbons.

Solute	Quencher	K_{oc} (L/g C)	Reference
Pyrene	Soil Humic Acid	170	Gauthier et al.
Phenanthrane	Soil Humic Acid	500	Gauthier et al.
Anthracene	Soil Humic Acid	850	Gauthier et al.
Pyrene	Soil Fulvic Acid	120	Gauthier et al.
Naphthalene	Soil Water Soluble Organics w/ Na	77	Traina et al.
1-Naphthol	Soil FA and HA	10-57	Current study

($\Delta S > 0$, $\Delta H \approx 0$) hydrophobic association, similar to other hydrophobic compounds (Chiou et al.,1983, 1986, 1987; Gauthier et al., 1987; Traina et al., 1989). Although a change in ionic strength may impose some effects on the HA molecules whose carboxylic groups were probably dissociated at pH 7, 1-naphthol was not charged at this pH and its behavior would not be expected to change significantly with ionic strength. Similar studies have shown virtually no effects of ionic strength on the complexation of organic solutes by dissolved humic substances (Gauthier et al., 1987; Means and Wijayaratne, 1982; Traina et al., 1989).

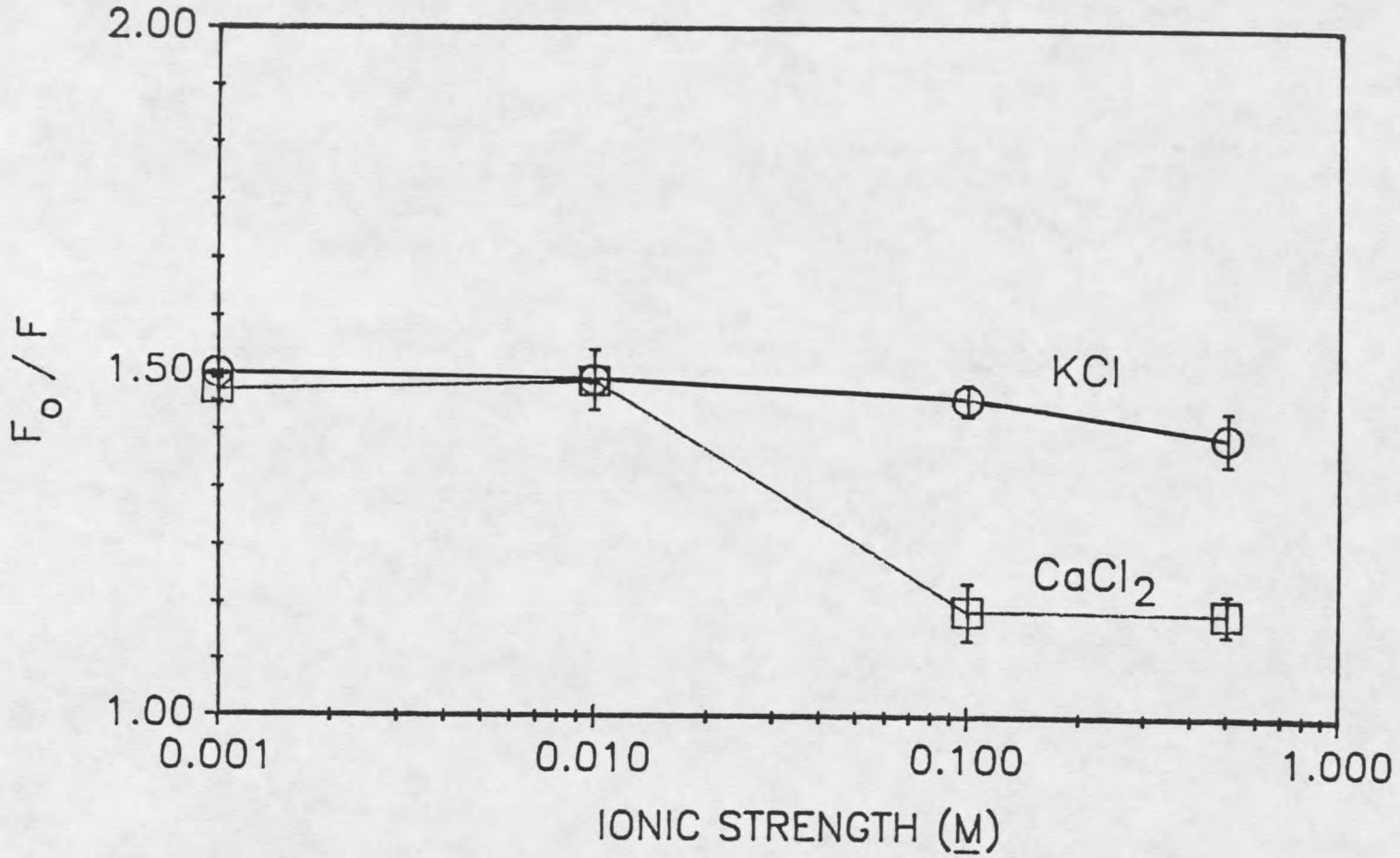


Fig. 4. Effects of solution ionic strength adjusted using KCl and $CaCl_2$ on the quenching ratio. [1-naphthol] = $1x10^{-5}$ *M*, IHSS [HA] = $2.00x10^{-3}$ *M* DOC.

2. pH

Acidification of the HA-naphthol solution to pH 3 lowered the quenching ratio slightly, while an increase in pH to 10 promoted the extent of association between 1-naphthol and HA (Table 5). This is consistent with the result of Lee and Farmer (1989) using the compound napropamide which has a fused ring structure similar to 1-naphthol. They found that a decrease in solution pH to 3.23 did not affect the association, but an increase in pH to 7.91 enhanced the association between napropamide and HA. In another study on complexation of naphthalene by water-soluble organic C, changes in pH values from 1.5 to 7.3 had no effect on binding (Traina et al., 1989). It is well known that the

Table 5. Effects of pH on the quenching ratio, F_o/F ([1-naphthol] = $1x10^{-5}$ *M*, IHSS [HA] = $2.00x10^{-3}$ *M* DOC).

pH	F_o/F
3.0	1.35±0.03
7.0	1.43±0.06
10.0	1.58±0.03

± Values are standard deviations of 3 replicates.

aggregation and dispersion of dissolved humic substances can be influenced by solution

parameters including pH (Ghosh and Schnitzer, 1980; Senesi et al., 1977; Underdown et al., 1985). Lee and Farmer (1989) explained the enhanced association with increasing pH as a result of an increase in dispersed humic polymer which provided more surface area to interact with napropamide. However, this explanation is not consistent with the results obtained with naphthalene, and suggests that other modes of interaction other than hydrophobic association may be involved. One association mechanism affected by pH is the interaction between the polar functional groups of the solute and the polymer. Hydrogen bonding and electron donor-acceptor processes have been suggested by several investigators (Senesi and Testini, 1983; Stevenson, 1972, 1982) to account for the interaction of herbicides with soil organic matter.

One additional mechanism which would result in greater binding with increasing pH is that at high pH, K^+ may be capable of forming a cation-bridge between dissociated humic acid functional groups and dissociated 1-naphthol. The effects of pH and ionic strength on the association reaction of dissolved humic substances with truly nonpolar and nonionic solutes are probably quite different, as evidenced in the report by Carter and Suffet (1982). They found that the association of DDT (2,2,-bis(4-chlorophenyl)-1,1,1-trichloroethane) with dissolved humic acid increased as the pH decreased or as the ionic strength or Ca^{2+} concentration increased. Additional studies would be necessary to understand the difference in effects of solution parameters on the complexation of nonpolar and polar organic solutes with dissolved humic substances.

The pK_a of the excited state 1-naphthol, 0.5 ± 0.2, is much lower than that of the ground state naphthol, 9.34 (Harris and Selinger, 1980; Weast, 1985). Thus, the

observed fluorescence (400-600 nm) of 1-naphthol in aqueous solutions at pH 7 was actually emitted by the naphthol anion. The quantum yield for the anion decreases rapidly with decreasing pH (Harris and Selinger, 1980; Webb et al., 1986). Our fluorescence intensity (F_o) data of free 1-naphthol in the absence of HA (not shown) were in good agreement with the pH effects on quantum yield. In the current study, 1-naphthol was equilibrated with HA prior to the fluorescence intensity measurements. Consequently, any change in the fluorescence intensity as a function of pH was a reflection of the remaining unbound naphthol in solution, and not a function of the pK_a of the excited 1-naphthol.

3. Metal Ion Bridging

Addition of Cu^{2+} and Zn^{2+} to the fluorophore solution without HA showed no significant change in the intensity of 1-naphthol fluorescence (Fig. 5 and 6). However, association of 1-naphthol with HA was enhanced at pH 9.5 as indicated by a 34.6% and 12.3% increase in the quenching ratio in the presence of Cu^{2+} and Zn^{2+}, respectively. At pH 5.0, there was no change in the quenching ratio. The pK_a for 1-naphthol is 9.34; at pH 9.5, more than 50% of the naphthol molecules were dissociated. Copper and Zn ions complex strongly with many ligands including HA, with Cu^{2+} usually showing a stronger affinity for carboxylic groups than Zn^{2+} (Martell and Smith, 1977). Thus, the observed effects can be explained in terms of cation bridging in which dissociated 1-naphthol anions are linked through Cu^{2+} or Zn^{2+} already in association with the functional groups of the humic polymer. This hypothesis is consistent with the fact that enhanced quenching was absent at pH 5.0, and the quenching was stronger for Cu^{2+} vs Zn^{2+} ions.

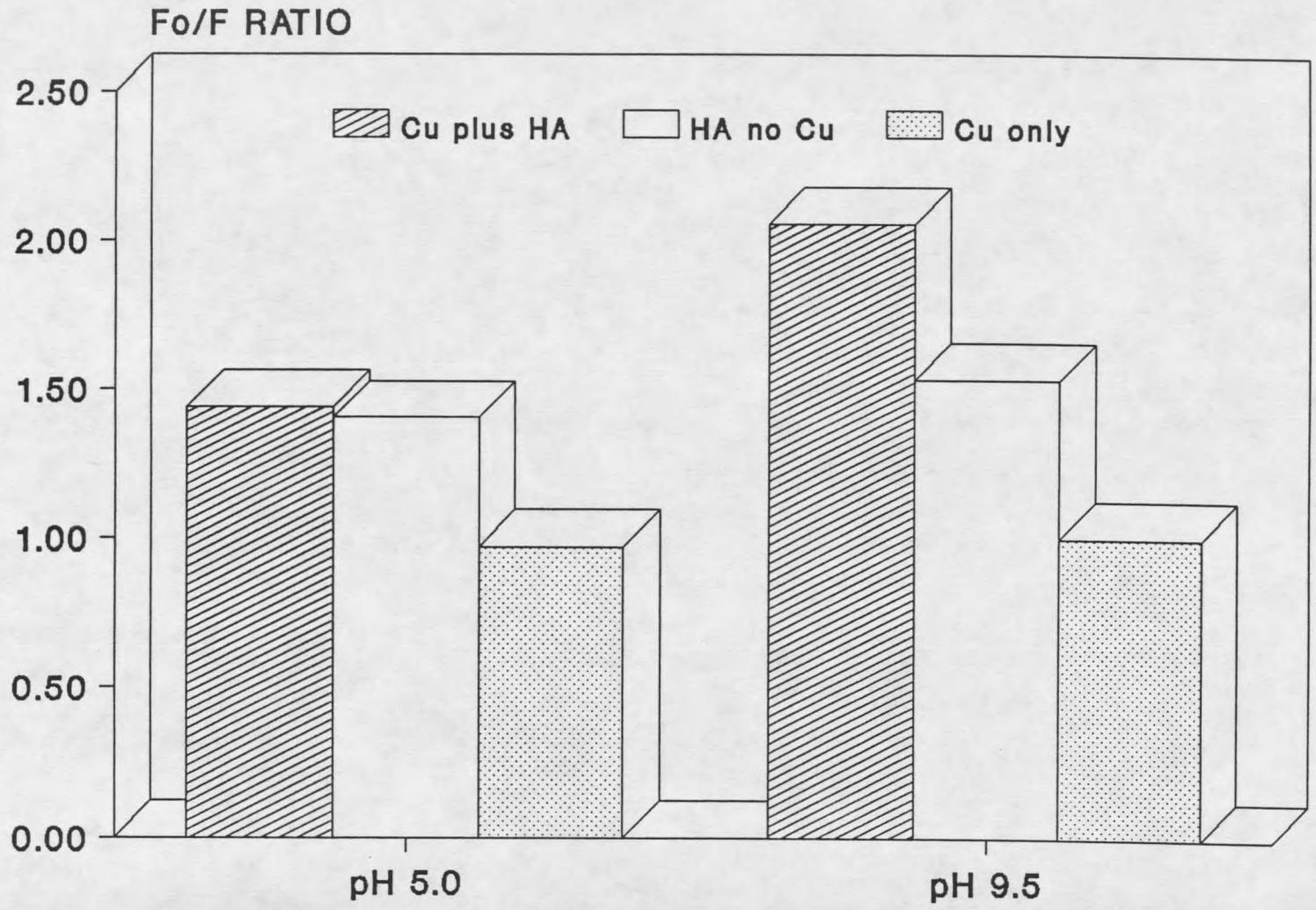


Fig. 5. Influence of solution pH and Cu (II) on the quenching ratio, F_o/F ([1-naphthol] = $1x10^{-5}$ *M*, IHSS [HA] = $2.00x10^{-3}$ *M* DOC, total [Cu^{2+}] = $1.00x10^{-4}$ *M*).

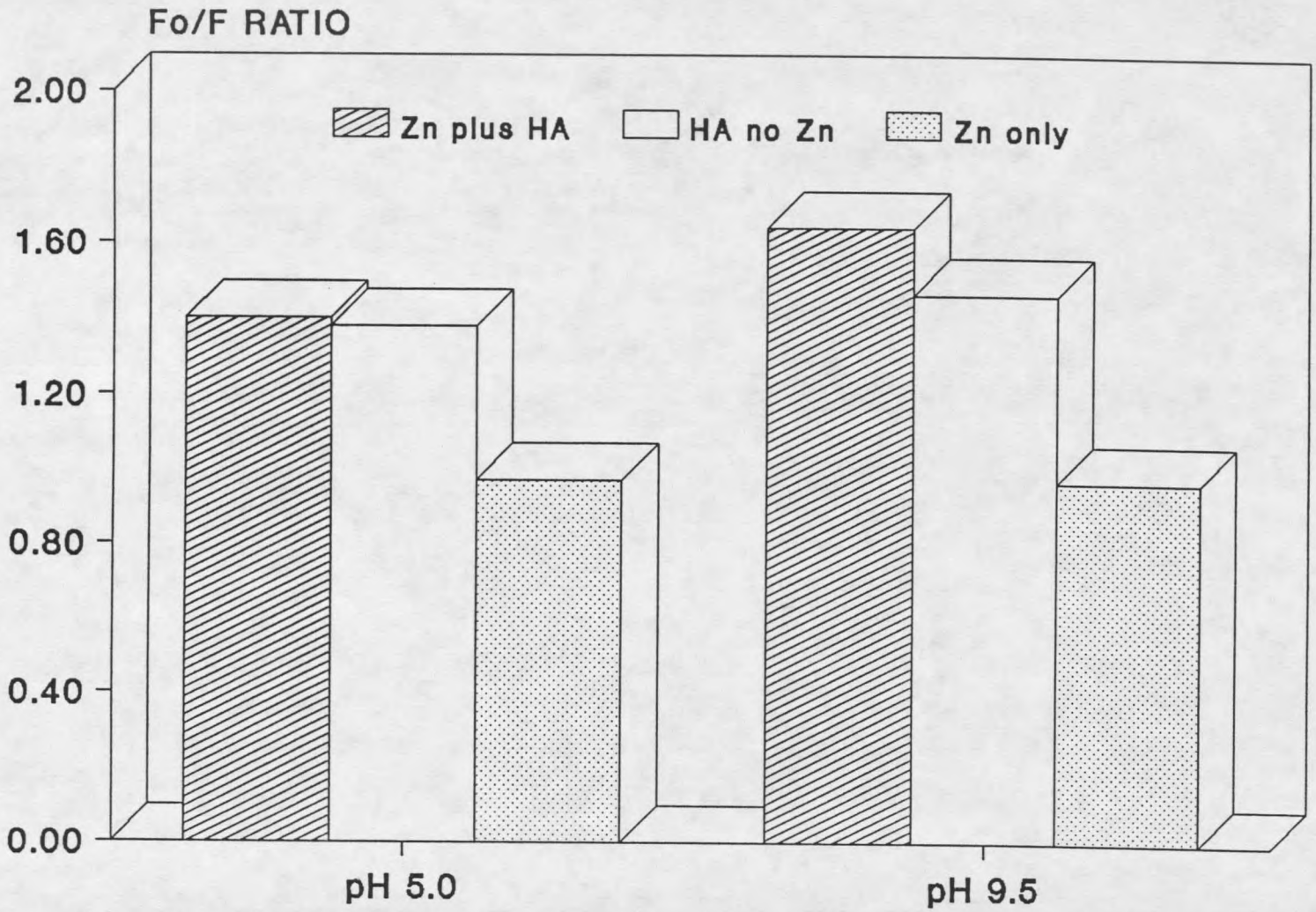


Fig. 6. Influence of solution pH and Zn (II) on the quenching ratio, F_o/F ([1-naphthol] = $1x10^{-5}$ *M*, IHSS [HA] = $2.00x10^{-3}$ *M* DOC, total $[Zn^{2+}] = 1.00x10^{-4}$ *M*).

Conclusions

Quenching of 1-naphthol fluorescence by HA appears to be primarily static. Generally, the conditional complexation constants for HA are larger than those for FA. Two binding mechanisms may exist for the complexation of 1-naphthol by HA and FA. The first mechanism involves hydrophobic interactions similar to partitioning between the solute and the hydrophobic subunits of HA and FA. The second mechanism is cation bridging to link the dissociated 1-naphthol with HA functional groups. The first mechanism is less dependent on pH and more dependent on the amount of hydrophobic sites of the polymer; thus HA provides more hydrophobic sites and shows more complexation than FA which is fairly hydrophilic. The second mechanism is pH-dependent and requires pH values near or above the pK_a of the organic solute.

The ionic strength and cation compositions of the solution are less important in the first mechanism, but become important in the second mechanism as the pH of the solution increases. The efficiency of a cation in promoting the complexation at higher pH is determined by its ability to bind with the functional groups of HA polymer.

Using the association constants determined in this study, and assuming a [HA] of 4 m*M* DOC, about 53% of added 1-naphthol was present as water soluble humate complexes. Consequently, soluble organic C in soil solutions and natural waters commonly ranging from 1 to 2 m*M* C may be important in determining the behavior of 1-naphthol in these environments.

CHAPTER 3

FLUORESCENCE LIFETIME MEASUREMENTS OF FLUORANTHENE, NAPROPAMIDE AND 1-NAPHTHOL IN THE PRESENCE OF HUMIC ACID

Introduction

In the previous section, we suggested that the quenching mechanism of 1-naphthol fluorescence by humic acid was primarily static, resulting from formation of water soluble naphthol-humate complexes. However, there is still some uncertainty about the mechanism of fluorescence quenching. For example, in a study on the quenching of naphthalene and 1-naphthol fluorescence by a soil humic acid, Morra et al. (1990) found that the Stern-Volmer constants obtained from intensity data were 140 (for naphthalene) and 55 (for 1-naphthol) times larger than those obtained from lifetime data. If the quenching was dynamic (due to the collisions of fluorophores and quenchers) and not static (due to complexation of fluorophore with quencher), the Stern-Volmer constants from both intensity and lifetime data should be the same (Lakowicz, 1983). Despite the differences in intensity and lifetime data, Morra et al. concluded that both static and dynamic quenching occurred. Gauthier et al. (1986) carried out a fluorescence quenching study and obtained linear Stern-Volmer plots for several polycyclic aromatic hydrocarbons binding to dissolved humic materials. The excellent linear relationship supported their assumption that quenching was static, resulting from solute-humic complexation. Further studies on the quenching mechanism are needed to clarify the role of complexation due to soluble organic C constituents in natural waters.

Fluorescence lifetime measurements are useful for distinguishing dynamic from static quenching. A decrease in fluorescence lifetime of the fluorophore in the presence of a quencher indicates dynamic quenching (Lakowicz, 1983). Moreover, for dynamic quenching, the quenching ratio should increase as the temperature increases.

The objectives of this study were to measure fluorescence quenching and fluorescence lifetimes of napropamide (2-(1-naphthoxy)-N,N-diethylpropionamide), 1-naphthol and fluoranthene in the presence of an International Humic Substance Society (IHSS) reference soil humic acid.

Materials and Methods

Theoretical

In Chapter 2, we showed that fluorescence quenching can be described by the Stern-Volmer equation:

$$F_o/F = 1 + K_{sv}[Q] \qquad [1]$$

where F_o and F are the fluorescence intensities in the absence and presence of the quencher Q, [Q] is the concentration of quencher, and K_{sv} is the Stern-Volmer constant. For a complexation reaction

$$F + Q = F\text{-}Q \qquad [2]$$

the conditional complexation stability constant K is

$$K = [F\text{-}Q]/([F][Q]) \qquad [3]$$

where [F] and [F-Q] are the concentrations of the free and the complexed fluorophore respectively. It was also shown that for static quenching,

$$K_{sv} = K = [F\text{-}Q]/([F][Q]) \quad [4]$$

and for collisional quenching, the Stern-Volmer constant is the product of the bimolecular quenching constant, k_q, and the fluorescence life time, τ_0, of the fluorophore in the absence of the quencher:

$$K_{sv} = k_q\tau_0 \quad [5]$$

The bimolecular quenching constant k_q is a function of the diffusion coefficients of the quencher and fluorophore (Chapter 2). Since diffusion coefficients generally increase with increasing temperature, the bimolecular quenching constant should also increase at higher temperatures. Utilizing the relationship between k_q, diffusion coefficients, temperature and viscosity of water, the following relationships can be derived to relate the quenching ratio (F_o/F) at 313.2 K to the quenching ratio at 283.2 K:

$$(F_o/F)_{313.2\,K} \approx 1.999(F_o/F)_{283.2\,K} - 0.999 \quad [6]$$

$$(F_o/F)_{315.2\,K} \approx 1.680(F_o/F)_{283.2\,K} - 0.680 \quad [7]$$

$$(F_o/F)_{313.2\,K} \approx 2.018(F_o/F)_{283.2\,K} - 1.018 \quad [8]$$

for 1-naphthol, napropamide and fluoranthene, respectively. Eq.'s [6], [7] and [8] indicate the predicted temperature dependence of dynamic quenching, and can be used as one criterion to distinguish static from dynamic quenching.

In addition, dynamic quenching is characterized by a decrease in the fluorescence lifetime (τ) of the fluorophore in the presence of quencher (Lakowicz, 1983):

$$\tau_0/\tau = 1 + k_q\tau_0[Q] \quad [9]$$

Thus, for dynamic quenching, the slope of the τ_0/τ vs [Q] plot should be equal to the slope of the F_o/F vs [Q] plot. If the slopes from these two plots are not equal, then either

the quenching is static or the distance between the excited fluorophore and the quencher is too close to allow effective diffusion and collision to occur before the excited fluorophore returns to the ground state and emits a fluorescence photon. Consequently, the measurement of fluorescence lifetimes in the presence of quencher is the most definitive method to distinguish static and dynamic quenching.

Detailed information on the theoretical and practical aspects of the pulse sampling method for the measurement of fluorescence lifetimes can be found elsewhere such as Lakowicz's work (1983). For our purpose, a brief summary of the photon-counting method is given as follows:

The sample is excited with a laser pulse, and the detection system measures the time between this pulse and the arrival of the first photon. The time between the excitation pulse and arrival of the first photon is measured for a large number of photons, and the distribution of arrival times represents the decay curve.

For a single fluorophore which decays exponentially,

$$F(t) = F_o' e^{-t/\tau} \qquad [10]$$

where t is the time, F(t) is the time-resolved decay of fluorescence intensity, and F_o' is the fluorescence intensity at $t = 0$. In instances where the decay curve is not adequately described by a single exponential, the observed decay is generally fitted to a sum of exponentials:

$$F(t) = \sum_i \alpha_i e^{-t/\tau_i} \qquad [11]$$

where α_i is a preexponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i.

Time-resolved decay curves of fluorescence anisotropy can also be measured using the above method. Polarizers are used to select the appropriate polarized components for the excitation and emission. Time-resolved anisotropy (R(t)) can then be calculated with the following equation (Lakowicz, 1983):

$$R(t) = (I_{\parallel}(t) - I_{\perp}(t))/(I_{\parallel}(t) + 2I_{\perp}(t)) \qquad [12]$$

where $I_{\parallel}(t)$ and $I_{\perp}(t)$ are the parallel and the perpendicular components of the emission.

In some cases, the fluorophore can be quenched both by collisions and complex formation with the same quencher. The characteristic feature of the Stern-Volmer plots in such circumstances is an upward curvature, concave towards the y axis. A modified form of the Stern-Volmer equation can describe such plots (Lakowicz, 1983):

$$F_o/F = (1 + K_D[Q])(1 + K[Q]) = 1 + (K_D + K)[Q] + K_DK[Q]^2 \qquad [13]$$

where $K_D = k_q\tau_0$, and $K = [F\text{-}Q]/([F][Q])$. In order to partition the dynamic and the static components, solutions to Eq. [13] are necessary. Letting $(K_D + K) = S$ and $K_DK = U$ and solving for K, we have

$$K^2 - SK + U = 0 \qquad [14]$$

and

$$K_D = S - U \qquad [15]$$

Since Eq. [14] is quadratic, two solutions to K and K_D are possible. The dynamic component, K_D, can generally be selected by the temperature dependence or lifetime shortening information.

Experimental

1. 1-Naphthol, napropamide and fluoranthene stock solutions

Solutions of 1-naphthol, napropamide and fluoranthene were prepared by dissolving high purity 1-naphthol (> 99 % pure, Aldrich Chemical Co., Milwaukee, WI), napropamide (99.9 % pure, USEPA Pesticides & Industrial Chemicals Repository, Research Triangle Park, NC) and fluoranthene (98 % pure, Aldrich Chemical Co.) into spectrum grade methanol (EM Science, Cherry Hill, NJ). The solutions were transferred to glass bottles wrapped with aluminum foil and stored at 5° C. Since these stock solutions contained methanol and contributed a final methanol concentration of 0.1% (v/v) to treatments that received these compounds, methanol was added to treatments that did not receive these compounds so that the final concentration of methanol was 0.1%. Preliminary studies showed that the presence of 0.1% (v/v) methanol did not alter the fluorescence of 1-naphthol, napropamide and fluoranthene.

2. Dissolved humic substances solutions

An IHSS reference soil HA (1R106H) was used in the fluorescence experiments. Some chemical properties of this HA can be found in Table 1, Chapter 2. A stock solution of this HA was prepared by dissolving the solid phase in dilute KOH (pH $\approx$ 8 - 9), then adjusting the pH to 7.0. The total dissolved organic carbon (DOC) of the stock solutions was measured with a Dohrmann DC-80 carbon analyzer (Dohrmann, Santa Clara, CA).

3. Fluorescence quenching experiments

A typical fluorescence experiment with these organic solutes consisted of 3

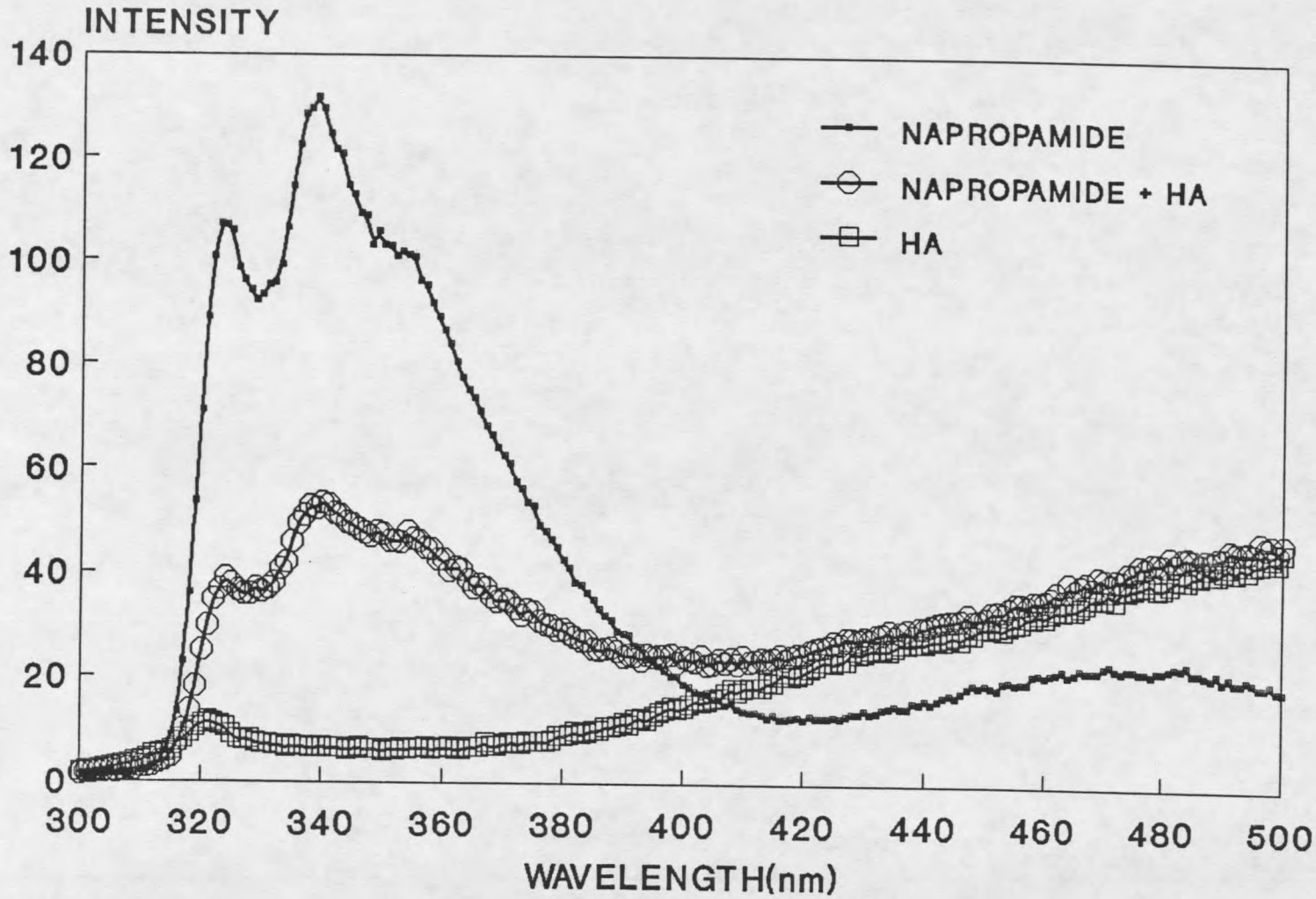


Fig. 7. Emission spectra of napropamide, napropamide plus IHSS humic acid (HA) and IHSS HA alone. [napropamide] = 2 x 10^{-6} *M*, [HA] = 2 m*M* C.

treatments in triplicate: solute, solute plus HA, and HA alone (Fig. 7). Twenty-five-ml volumetric flasks were used as reaction vessels and at least 12 h was allowed for equilibration before fluorescence measurements. All experiments were performed in 0.01 *M* KCl at pH 7.0. All fluorescence measurements were made with a Spex fluorolog-2 spectrofluorometer (Spex Industries, Inc., Edison, NJ) equipped with a 150 W Xe lamp. All fluorescence intensity measurements were made with excitation wavelengths fixed at 287, 290 and 294 nm, and emission wavelengths fixed at 464, 339 and 468 nm for fluoranthene, napropamide and 1-naphthol, respectively. Ten scans were made for each intensity measurement.

The inner filter effect was corrected for each measurement by manually adjusting the cuvette position via an x-y translation stage. The position of the micrometer was recorded for the solute plus HA treatment and used for the intensity measurement of the HA blank treatment. The effective bandpass was 2.25 nm and 4.5 nm for the excitation monochromator and the emission monochromator, respectively.

4. Temperature experiments

A brass water-jacketed cuvette holder was used to make fluorescence measurements at temperatures ranging from 283 K to 313 K ($\pm 1^0$). The concentrations of 1-naphthol, napropamide and fluoranthene were fixed at 1.0, 2.0 and 1.0 μM, respectively, while the concentration of HA was fixed at 2.0 m*M* DOC.

5. Fluorescence lifetime measurements

The fluorescence decay curves were obtained as a function of quencher (HA in 0.01 *M* KCl, pH 7) concentration ranging from 0 to 1.5 m*M* C using a time-correlated

single photon counting (TCSPC) apparatus (Regional Laser and Biotechnology Laboratories, Univ. of Pennsylvania). The initial concentrations of fluoranthene, 1-naphthol and napropamide were 1.0, 10.0 and 2.0 μM respectively. A Nd:YAG laser was used to power a dye laser whose 580 nm output was converted to 290 nm --- the excitation wavelength for all 3 compounds. The excitation pulse width was 10 ps at a frequency of 4 MHz (250 ns). A monochromator was placed in the front of the photo-multiplier tube (PMT) to select the emission wavelength. The response time of the PMT was 25 ps. A special 0.3 ml cuvette was used for 1-naphthol while a flow cell was used for napropamide and fluoranthene to avoid fast photodegradation and subsequent depletion of these two compounds in a small volume cuvette. Data acquisition time varied depending on the fluorescence intensity. The collected fluorescence decay curves were analyzed with a computer program called LIFETIME for curve-fitting and lifetime calculations.

6. Time-resolved fluorescence decays of anisotropy experiments

To obtain the time-resolved anisotropy decay curves of 1-naphthol and napropamide fluorescence in the presence and absence of HA, rotating polarization filters were used to alternatively select the parallel and the perpendicular components of the emission; all other experimental procedures were identical to the lifetime measurements.

Results and Discussion

Stern-Volmer plots of the quenching of fluoranthene and 1-naphthol fluorescence by the IHSS HA were essentially linear from 0 - 4.0 mM C (Fig. 8). The Stern-Volmer

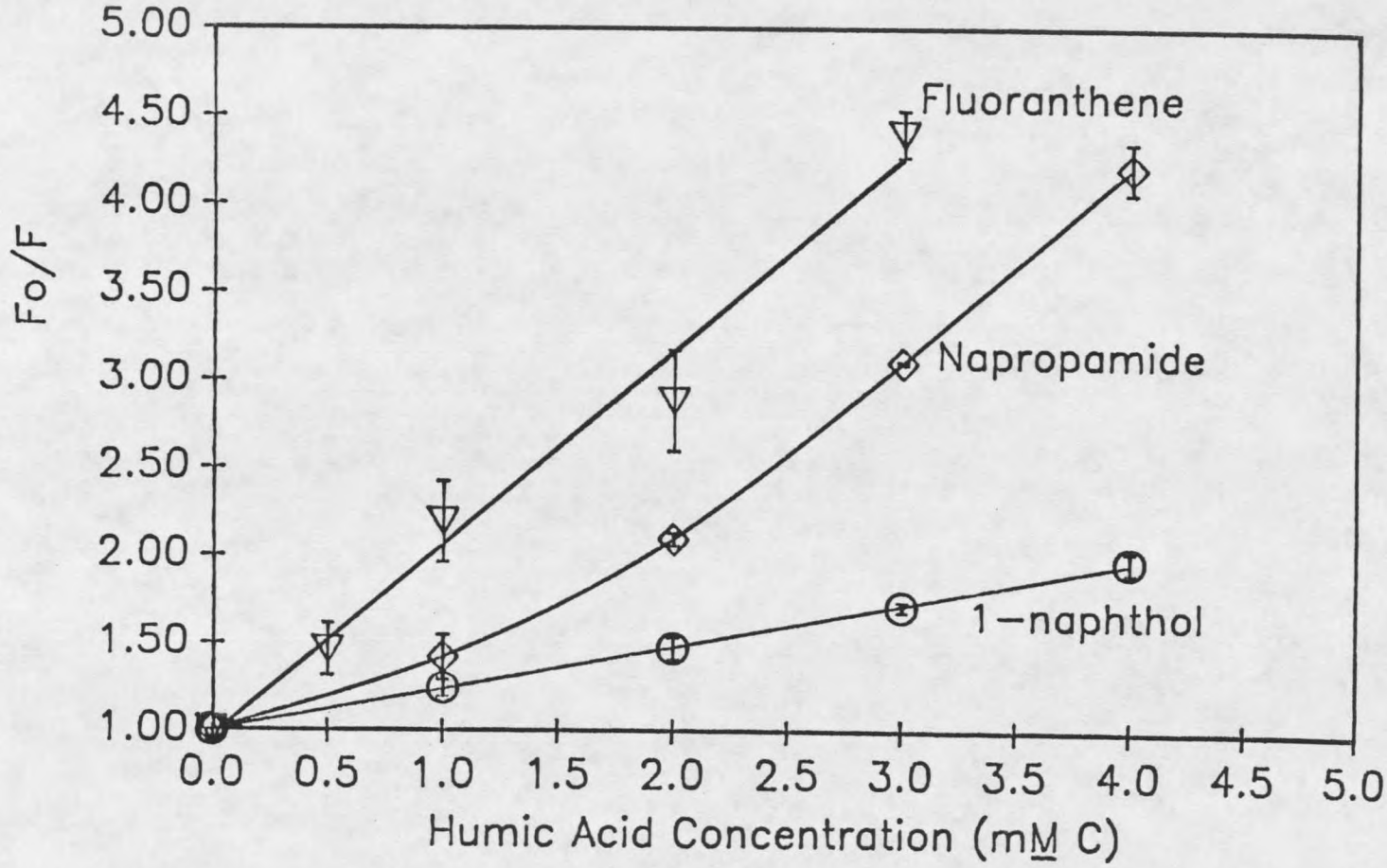


Fig. 8. Quenching of fluoranthene, 1-naphthol and napropamide fluorescence by IHSS reference soil humic acid.

plot for napropamide is slightly concave towards the y-axis, indicating a small dynamic component. Non-linear regression analysis of the napropamide data resulted in the following equation describing the quenching of napropamide fluorescence by HA:

$$F_0/F = 0.99 + 322[HA] + 12207[HA]^2 \quad [16]$$

The correlation coefficient (r) was 0.9997, showing excellent fitting to Eq. [12]. Using Eq.'s [13] and [14], two conditional complexation constants (K) were obtained: 3.6 and 23.2 L/g C. Data from the temperature and fluorescence lifetime experiments (discussed below) support the assumption that the quenching of napropamide fluorescence by HA was primarily static, consequently, it is more appropriate to choose 23.2 L/g C as the conditional stability constant for the complexation of napropamide with HA. Assuming that the HA had an average molecular weight of 5000 daltons, and using the smaller K for napropamide, the calculated bimolecular quenching constant (k_q) was still larger that the upper limit ($1x10^{10}$ $M^{-1}s^{-1}$) of k_q for aqueous solutions. This is further support for a static quenching mechanism. The Stern-Volmer constants (complexation stability constants if quenching is static) for fluoranthene and 1-naphthol were 91.5 and 20.1 L/g C, respectively. The reported conditional equilibrium complexation constants for polycyclic aromatic hydrocarbons binding to dissolved soil HA ranged from 170 for pyrene to 850 L/g C for anthracene (Gauthier et al., 1986). For binding of naphthalene to soil water soluble organic with Na^+, the constant was 77 L/g C (Traina et al., 1989). Thus, the conditional complexation equilibrium constants obtained in this study were in good agreement with other fluorescence quenching studies. Since the mechanism of complexation between nonionic organic solutes and dissolved humic substance is

generally hydrophobic association, complexation constants should increase with increasing hydrophobicity of the solute. Our data showed increasing K values in the order fluoranthene > napropamide > 1-naphthol, consistent with the hydrophobicity of these solutes as inferred from their solubilities in water.

Temperature ranging from 283 to 313 K had very little effect on the quenching ratio (F_o/F) of fluoranthene, napropamide and 1-naphthol (Fig. 9). Predicted quenching ratios at 313 K (315 K for napropamide) assuming a dynamic quenching mechanism (Eq.'s [6] - [8]) were 5.34, 3.74 and 3.15 for fluoranthene, 1-naphthol and napropamide, respectively. The measured quenching ratios at 313 K were much smaller than the theoretically predicted dynamic quenching ratios, suggesting that the primary quenching mechanism was not dynamic. The slight increase in quenching ratio for napropamide with temperature may be due to the presence of a small dynamic component or due to the thermal instability of napropamide-humate complexes. Lifetime data (discussed below) tend to support the latter because the lifetime did not shorten in the presence of HA, however, the Stern-Volmer plot seems to indicate the presence of a small dynamic component. From the temperature and lifetime information, we conclude that the primary mechanism of the quenching of napropamide fluorescence by the HA was due to complex formation.

Multiexponential fluorescence decay curves were observed for fluoranthene with and without HA (Fig. 10). The multiple decays were thought to be mainly caused by impurities (about 2 % impurities in the solid phase of fluoranthene) in the solution, but polarization excitation of the fluorophore may also contribute to multiple decay since the

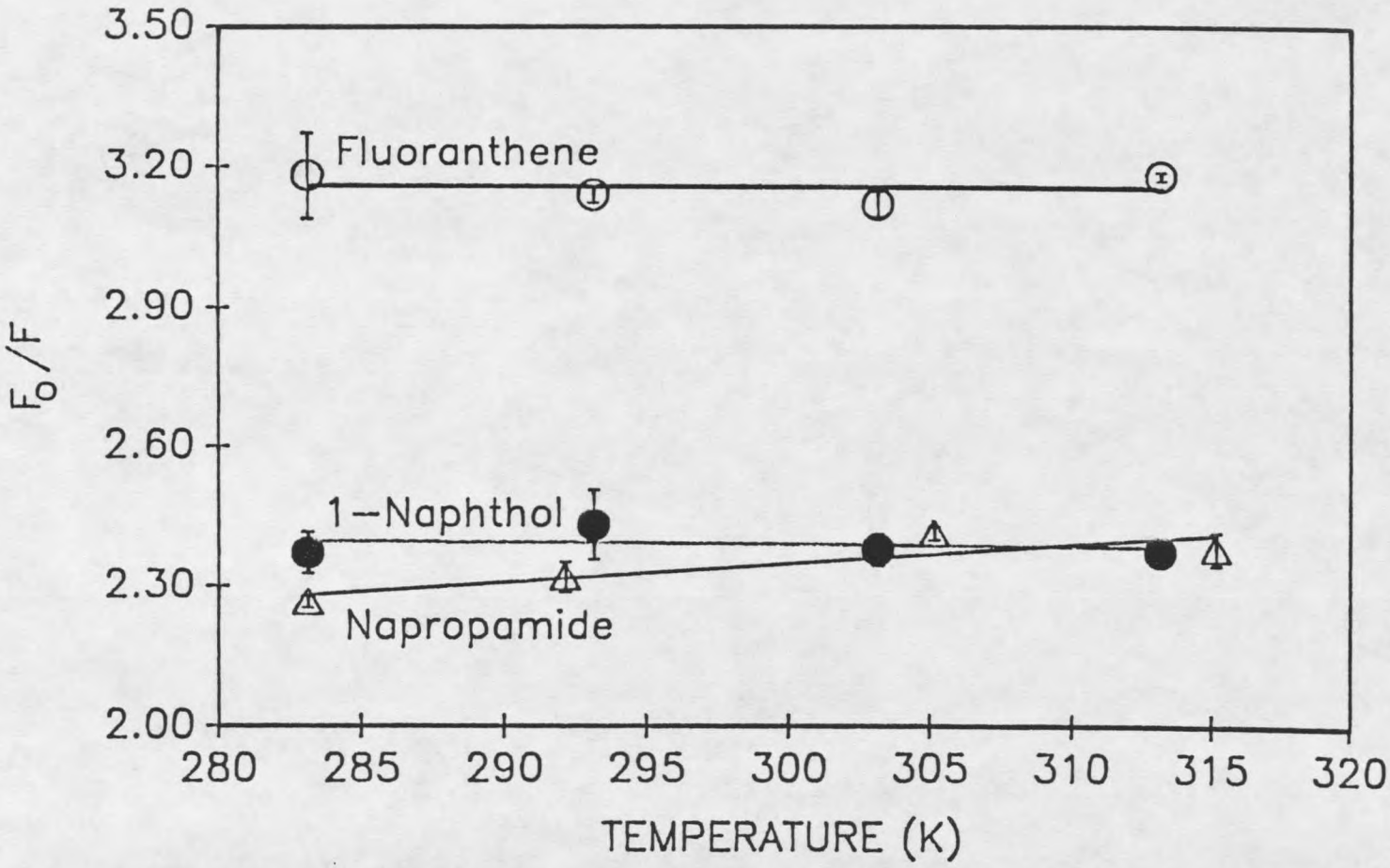


Fig. 9. Effects of temperature on quenching of fluoranthene, 1-naphthol and napropamide fluorescence by IHSS reference humic acid (HA). [HA] = 2 m*M* C.

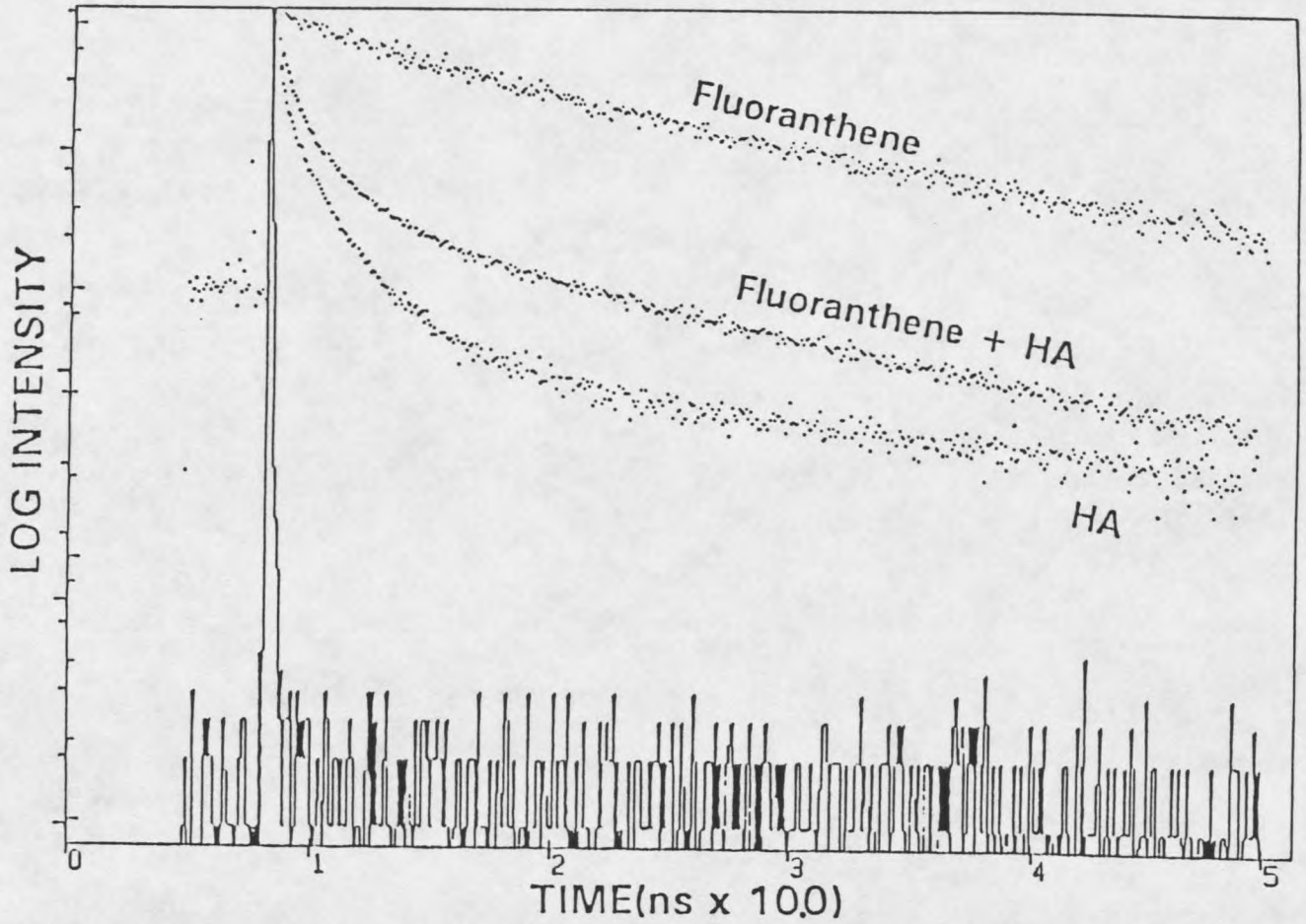


Fig. 10. Fluorescence decay curves of fluoranthene, fluoranthene plus IHSS humic acid (HA) and HA alone.

laser excitation beam was highly polarized. Multiexponential decays of fluorescence can be observed even for a single fluorophore which decays exponentially, if a particular polarized component is selectively observed (Lakowicz, 1983). In the absence of fluoranthene, the HA fluorescence decay curves (not shown) showed steeper slopes with increasing HA concentration. The parallel property for the majority of the fluoranthene and fluoranthene + HA curves can be easily seen, and this similarity of fast decay at the beginning parts of the fluoranthene + HA and HA curves indicates the strong contribution of HA fluorescence. Since the excitation pulses indiscrimately excited both the fluoranthene and HA molecules, the fluorescence emission maximum of fluoranthene almost overlapped that of HA fluorescence. A similar problem existed for the 1-naphthol + HA curve (Fig. 11). Thus, it is necessary to eliminate the HA contribution to the fluorescence decay curves of fluoranthene and 1-naphthol in the presence of HA. The lifetimes for fluoranthene and 1-naphthol in the presence of HA reported in this study resulted from analyses that eliminated the HA contribution to the fluorescence decay curves.

Since the response time of a PMT determines its ability to accurately measure the decay curves, a slower PMT may not be able to record the fast fluorescence decays of HA and thus be unable to distinguish the HA contribution. Failure to consider the HA contribution can significantly influence fluorescence decay curves, leading to erroneous interpretations. Therefore, the small but detectable slopes (< 0.0005) of the τ_0/τ vs dissolved HA concentration for the quenching of naphthalene and 1-naphthol fluorescence obtained in the study by Morra et al. (1990) probably resulted from a slow PMT and

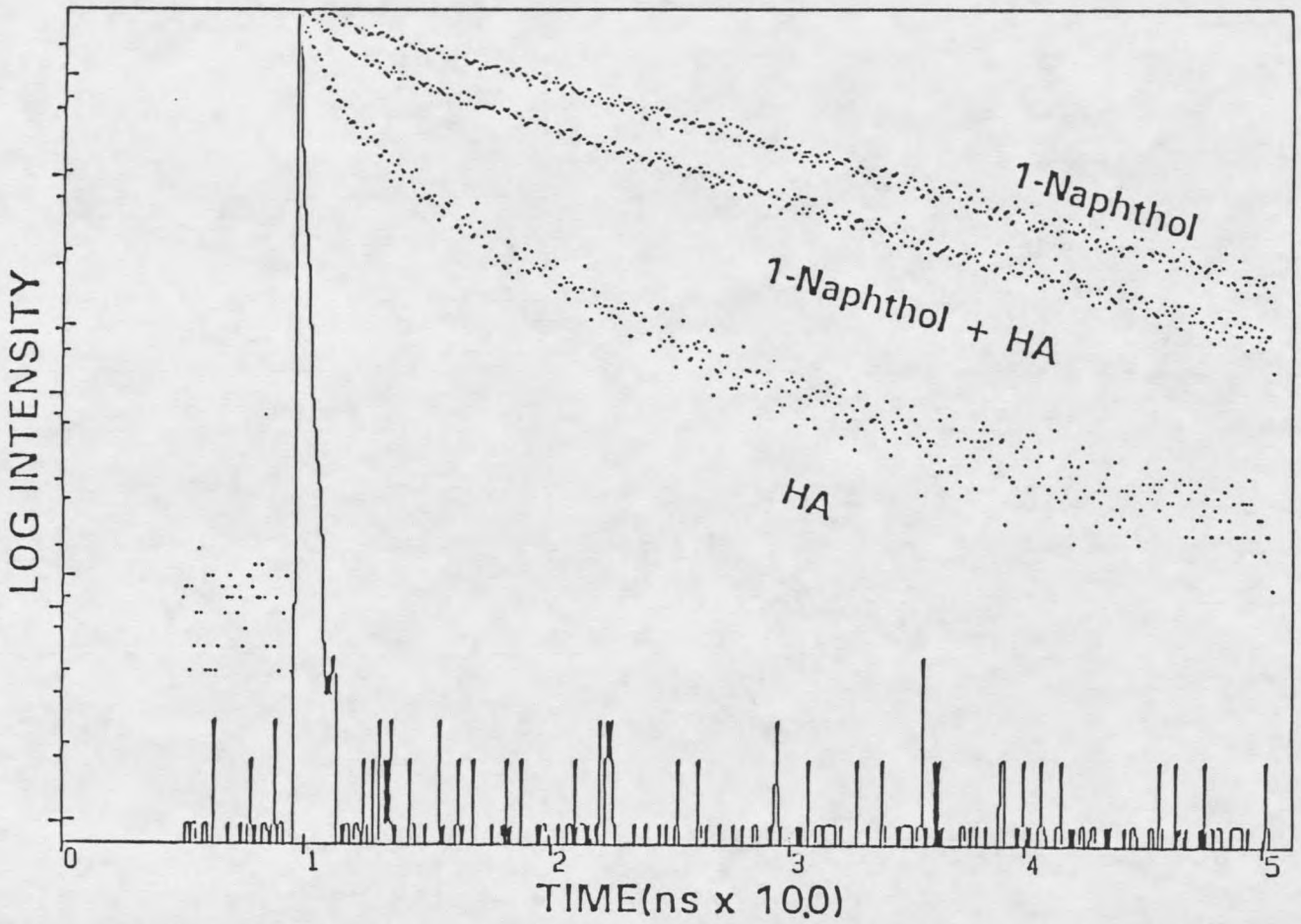


Fig. 11. Fluorescence decay curves of 1-naphthol, 1-naphthol plus IHSS humic acid (HA) and HA alone.

negligence of HA interference. It should be pointed out that the large discrepancy between the slopes (Stern-Volmer constants) of the F_0/F vs HA concentration and the τ_0/τ vs HA concentration plots in the study by Morra et al. (1990) probably reflect a static quenching mechanism, because for dynamic quenching, these slopes should be equal (Eq.'s [1], [5] and [9]).

It is worthwhile to note that the weak fluorescence emitted by the HA had multiple lifetimes, with the majority (60 %) shorter than 1 ns. Such short fluorescence lifetime makes the HA a good photo-protector in natural systems and may be important in protecting UV-sensitive compounds.

The linear property of the fluorescence decay curves for napropamide and napropamide + HA indicated a single exponential decay (Fig. 12). Since the emission maximum for napropamide fluorescence is far from the HA fluorescence band, the HA contribution was negligible with a monochromator.

In the absence of the HA quencher, the fluorescence lifetimes for fluoranthene, napropamide and 1-naphthol in 0.01 *M* KCl were 43.8, 3.27 and 8.03 ns, respectively (Fig. 13). The fluorescence lifetimes of 1-naphthol and napropamide did not change significantly over a range of humic acid concentrations (Fig. 13). Fast fluorescence decay contributed from HA was significant for fluorescence lifetime measurements of fluoranthene (Fig. 10). However, the lifetimes did not change with increasing HA concentrations (Fig. 13). There was a decrease in fluoranthene fluorescence lifetime from no HA to 0.3 m*M* C HA (Fig. 13). The multiple exponential decay (Fig. 10) of the

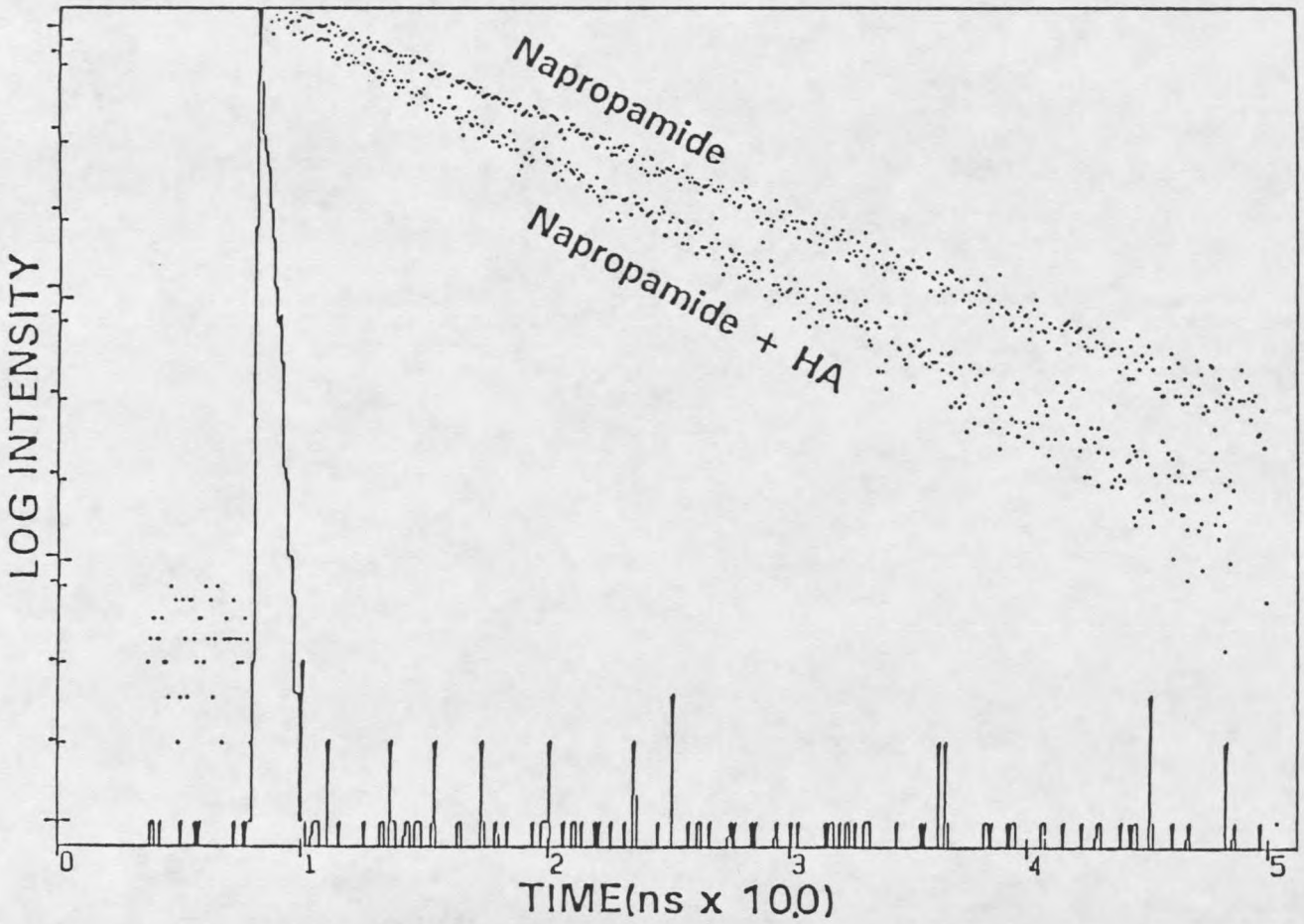


Fig. 12. Fluorescence decay curves of napropamide and napropamide plus humic acid (HA).

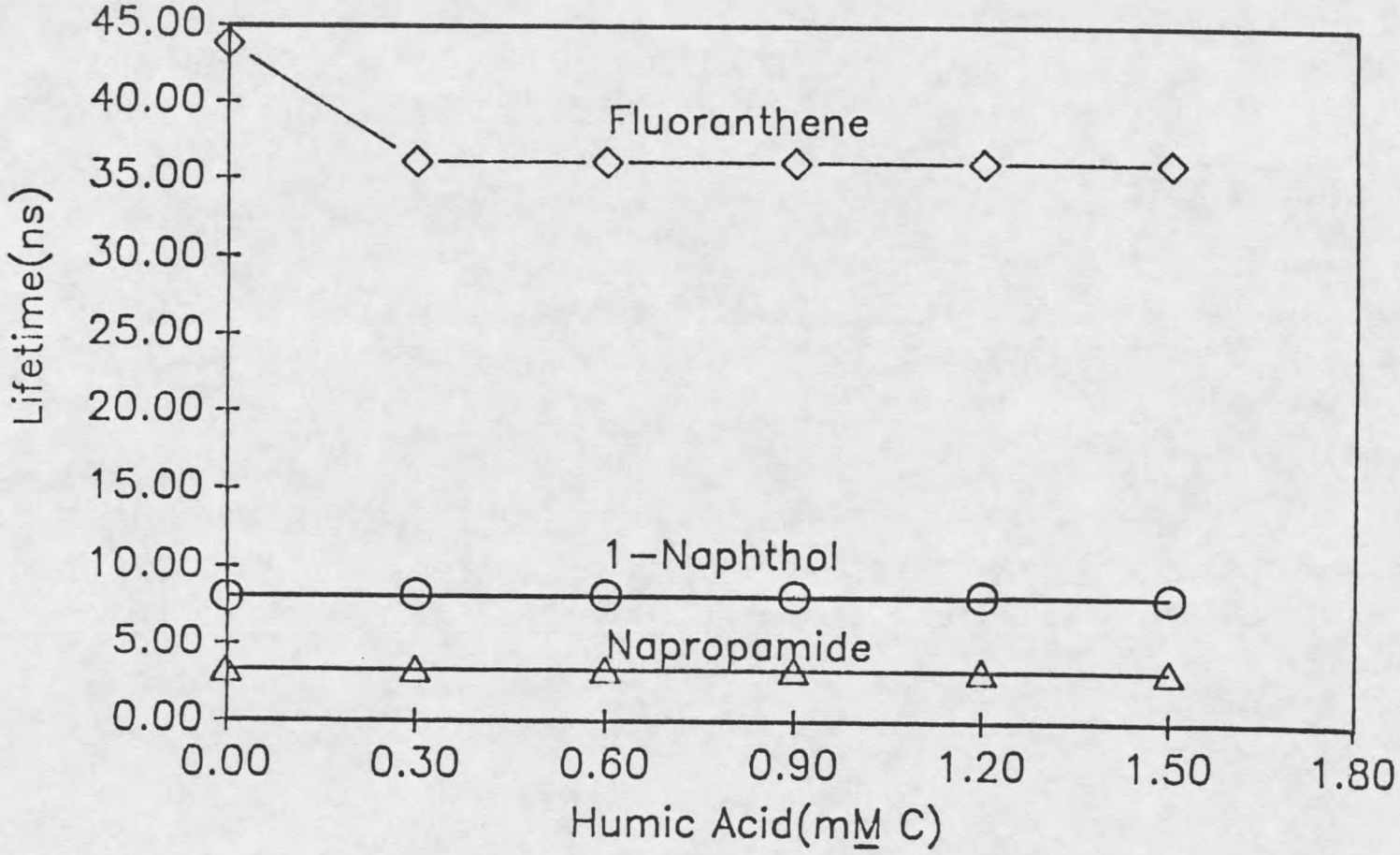


Fig. 13. Fluorescence lifetimes of fluoranthene, 1-naphthol and napropamide in the absence and presence of IHSS reference humic acid.

fluoranthene curve, plus the fact that the LIFETIME program emphasizes the beginning part of the decay curve may explain such a decrease in lifetime.

In cases where the bound fluorophore is fluorescent, the fluorophore may show time-resolved anisotropy decay. No significant time-resolved anisotropy decays of napropamide and 1-naphthol fluorescence were observed in the absence (Fig. 14) and presence of HA (Fig. 15). This result is consistent with the study of steady state fluorescence anisotropy of 1-naphthol by Morra et al. (1990), and may be explained by several reasons. Assuming the fluorophore-humate complexes were nonfluorescent, fluorescence decays would only originate from the free fluorophores. Since the fluorophore did not exhibit any time-resolved anisotropy in the absence of HA, then no time-resolved anisotropy decays should be observed in the presence of HA. Very fast depolarization decay, beyond the response time of the PMT, may explain why the solutes did not show anisotropy decays. Such fast depolarization may be the result of two excited states residing at the same excitation wavelength. Again, data on the time-resolved fluorescence decays of anisotropy supported the assumption that the quenching of 1-naphthol and napropamide fluorescence by the HA was static.

Conclusions

The average fluorescence lifetimes for fluoranthene, napropamide and 1-naphthol in 0.01 *M* KCl were 43.80, 3.27 and 8.03 ns, respectively. The fluorescence lifetimes did not decrease with increasing HA concentration, indicating the presence of a static

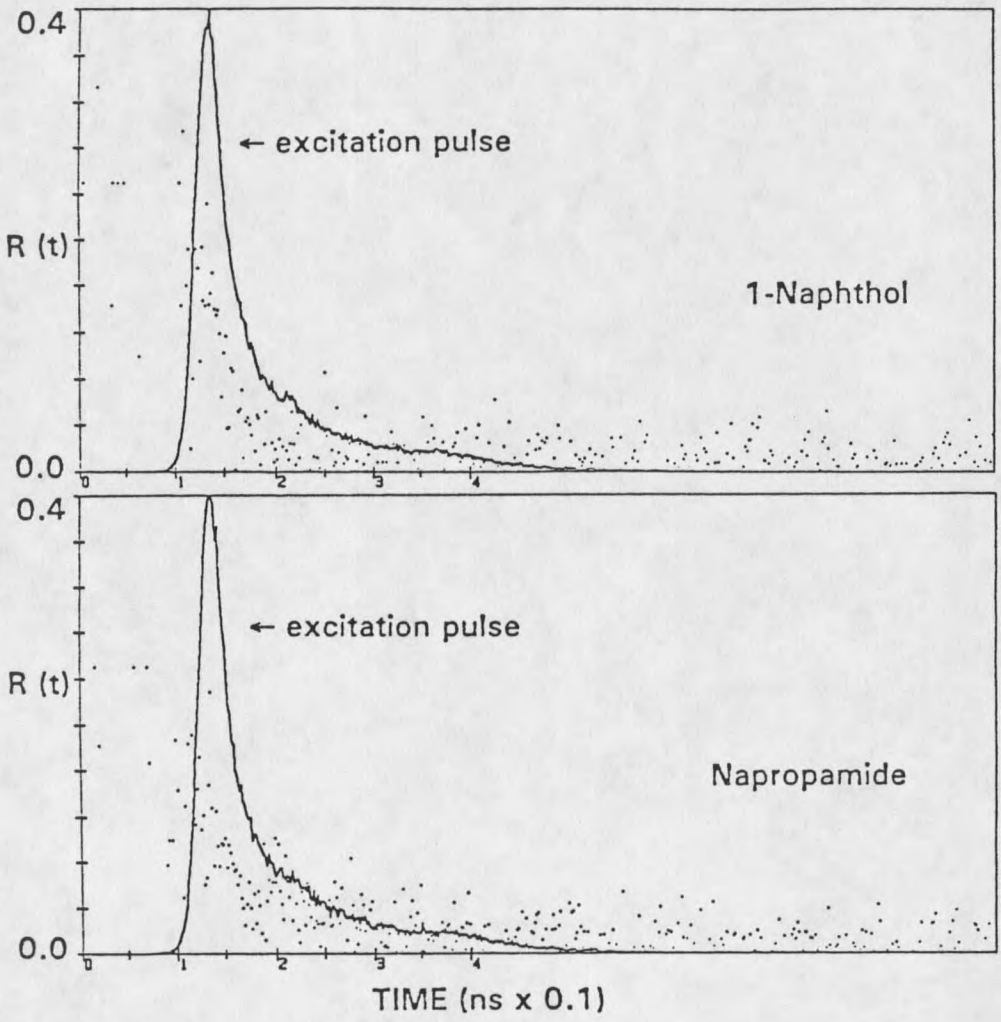


Fig. 14. Anisotropy (R(t)) fluorescence decays of 1-naphthol and napropamide in 0.01 *M* KCl, pH = 7.0. No significant anisotropic decays were detected.

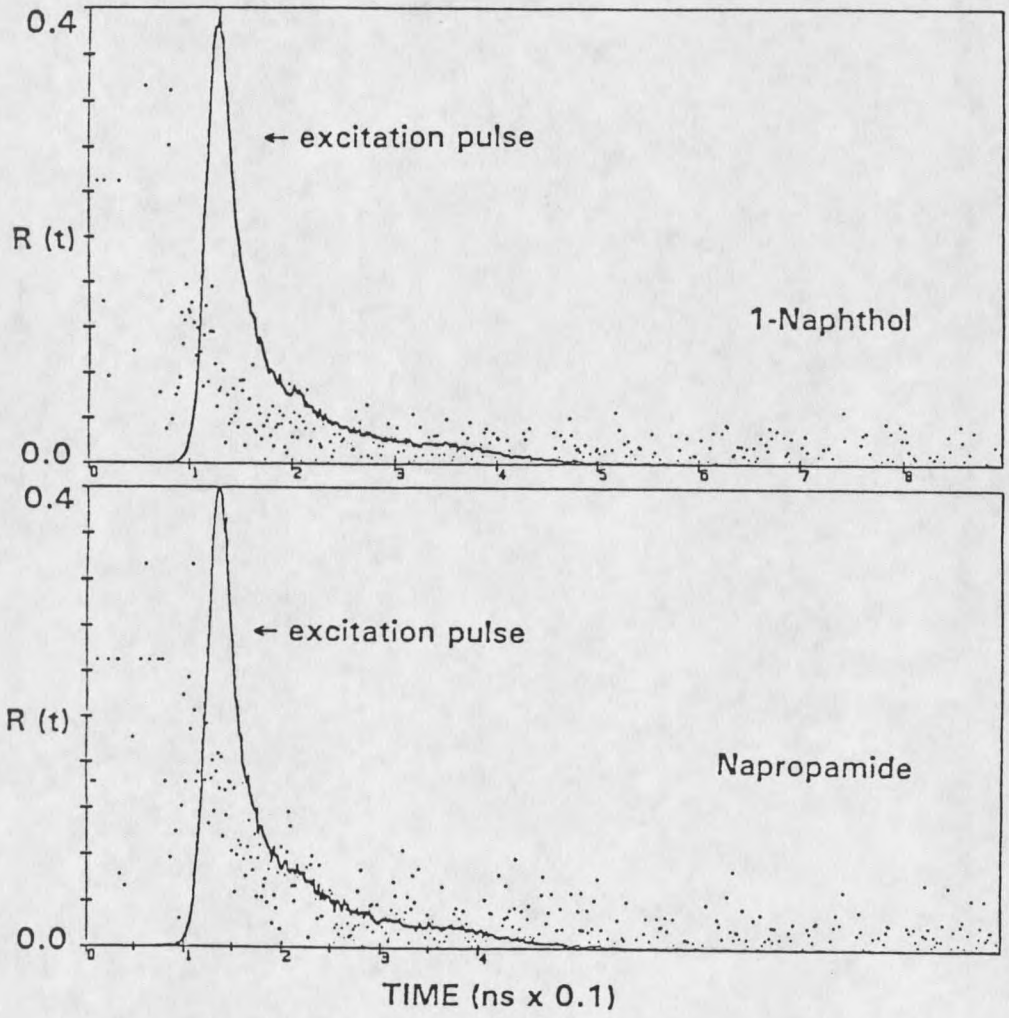


Fig. 15. Anisotropy (R(t)) fluorescence decays of 1-naphthol and napropamide in the presence of IHSS humic acid (HA) and 0.01 *M* KCl, pH = 7.0. No significant anisotropic decays were detected.

quenching mechanism. Quenching ratios did not increase significantly with increasing temperature, strongly suggesting the absence of dynamic quenching.

Decreased fluorescence intensity with no change in fluorescence lifetimes in the presence of HA, and no significant anisotropy fluorescence decays for napropamide and 1-naphthol in the presence or absence of HA, both suggested that the solute-humate complexes were nonfluorescent.

The complexation stability constants for fluoranthene, 1-naphthol and napropamide with IHSS reference HA 1R106H were 91.5, 20.1 and 23.2 L/g C, respectively. These values are consistent with other fluorescence studies and show increasing complexation with increasing hydrophobicity of the solutes.

CHAPTER 4

COMPLEXATION OF DICHLOBENIL, TRIALLATE AND TRIFLURALIN BY DISSOLVED HUMIC SUBSTANCES UTILIZING HEADSPACE GAS CHROMATOGRAPHY

Introduction

The binding (complexation) of hydrophobic organic solutes by dissolved organic carbon (DOC) may significantly influence the behavior and fate of these compounds in the environment. Complexation of hydrophobic organic solutes with DOC has been shown to (i) increase the apparent water solubility of these compounds (Chiou et al., 1986, 1987), (ii) decrease the solvent extractability and sorption onto particles (Hassett and Anderson, 1979, 1982; Gschwend and Wu, 1985), (iii) reduce the bioavailability and toxicity of the solutes (Dell'Agnola et al., 1981; Landrum et al., 1987), and (iv) change the rate of volatilization and bioaccumulation (Hassett and Milicic, 1985; Boehm and Quinn, 1976; Leversee et al., 1983). Although these studies have documented the formation of organic solute-humic complexes, there is still insufficient data to ascertain the importance of pesticide-humic complexes in natural systems.

One of the most important properties of volatile organic solutes is their Henry's law constant (K_h). Pesticides with widely differing K_h values have different volatilization and accumulation rates in soils (Spencer et al. 1988). For many compounds, the rate of volatilization from aqueous phases to the atmosphere is a function of K_h (Mackay et al., 1979). Unfortunately, the availability and accuracy of Henry's law constants for many organic pollutants and pesticides are limited. Attempts to use pure solute vapor pressure

and aqueous solubility to calculate K_h have achieved only limited success because of the unavailability of data and the errors associated with estimating vapor pressure and solubility at ambient temperature (Burkhard et al, 1985; Suntio et al, 1988). Efforts using quantitative structure-activity relationships (QSAR), such as molecular connectivity and polarizability, to predict K_h have resulted in moderate success with compounds of low molecular weight and simpler chemical structures (Arbuckle, 1983, Nirmalakhandan and Speece, 1988). With larger and more complex compounds, the ability of QSAR models to predict K_h deteriorates. Generally, the estimates of K_h using these two methods may vary tremendously, and it is not uncommon to have measured K_h values differing from predicted K_h values by several orders of magnitude. Thus, experimental measurements of K_h are necessary to obtain reliable constants for understanding potential reaction pathways of volatile organic compounds in natural systems.

Several techniques, including dynamic headspace (DHS, Mackay et al., 1979; Yin and Hassett, 1986), equilibrium partitioning in closed system (EPICS, Gossett, 1987; Yurteri et al., 1987) and wetted-wall column (WWC, Fendinger and Glotfelty, 1988 and 1990) have been used to determine Henry's law constants for a variety of compounds. DHS and WWC methods employ a mobile gaseous phase and unavoidably perturb other equilibria such as the complexation of a volatile organic solute by DOC. EPICS requires high vapor pressure in order to be accurate while most pesticides have very low vapor pressure at environmental temperatures. Static headspace analysis, when coupled with electron capture detection (ECD) gas chromatography (GC), is a very useful method for measuring Henry's law constants for many pesticides, even in the presence of DOC

(Dietz and Singley, 1979). More practical and theoretical details concerning the use of headspace GC analysis have been reviewed by Ioffe and Vitenberg (1984).

The objectives of this study were to (i) provide a method for simultaneously determining Henry's law constants and conditional complexation stability constants for herbicides dichlobenil (2,6-dichlorobenzonitrile), triallate (S-(2,3,3-trichloro-2-propenyl)-bis(1-methylethyl) carbamothioate) and trifluralin (2,6-dinitro-N,N-diprophyl-4-trifluoro-methylaniline) in the presence of dissolved humic substances, (ii) determine the extent of interaction among these solutes in the presence of humic acid (HA), (iii) and understand the effects of solute molecular structure, hydrophobicity and solution ionic strength on herbicide-HA complexation.

Materials and Methods

Stock solutions of dichlobenil, triallate and trifluralin were prepared by dissolving the reference standards of these herbicides (Pesticides & Industrial Chemicals Repository, USEPA, Research Triangle Park, NC. Purity was 99.9%, 99.9%, 99.5%, respectively) into spectrum-grade methanol (EM Science, Cherry Hill, NJ). Some properties of these compounds are summarized in Table 6.

Six different humic (HA) and fulvic acids (FA) were used in this study (Table 7). Stock solutions of these acids were prepared by dissolving the solid phase in dilute KOH (pH $\approx$ 8 - 9), then adjusting the pH to 7.0. The total DOC of the stock solutions was measured with a Dohrmann DC-80 C analyzer (Dohrmann, Santa Clara, CA).

Table 6. Properties of dichlobenil, triallate and trifluralin[†]

Herbicide	Molecular Weight	Melting Point (° C)	Vapor Pressure[‡] (mm Hg) at 25° C	Water Solubility (g/m^3)	log K_{ow}
Dichlobenil	172.02	144-145	0.0011-0.0032[§]	25	2.90
Triallate	304.70	29-30	0.00012	4	4.25[¶]
Trifluralin	335.29	46-47	0.00011	0.5	3.06

[†] Cited from Suntio et al., 1988.

[‡] From Herbicide Handbook, 3rd Ed., Weed Sci. Soc. Am.

[§] Extroplated from data given in the Herbicide Handbook.

[¶] Estimated from log K_{ow} = 5.00 - 0.670(log S), where S = aqueous solubility in μmol/L (Chiou et al., 1977)

Table 7. Chemical properties of humic substances used in the HSGC study.[†]

Humic Substance	C	H	O	N	S	P	Ash	COOH (mmol charge/g)	OH (mmol charge/g)	Percentage of Aromatic[§] C
	g kg^{-1}									
IHSS[¶] Reference HA (1R102H)	579.9	37.8	336.9	41.8	4.1	3.20	9.00	ND[‡]	ND	42
IHSS Reference HA (1R106H)	541.3	49.1	353.9	50.3	6.0	4.00	15.20	ND	ND	24
Aldrich HA	551.9	48.3	284.3	6.0	36.4	0.8	68.3	ND	ND	ND
Montana Soil HA	432.7	40.2	354.9	30.9	ND	ND	15.24	ND	ND	ND
IHSS Standard FA (1S101F)	537.5	42.9	404.8	06.8	0.5	0.01	0.82	6.0	1.2	18
Wheat Straw FA	555.0	52.0	357.0	08.0	ND	ND	2.40	5.5	0.7	30

[†] All items on a moisture-free basis

[‡] ND: not determined

[§] Percentage Aromaticity = $\frac{\text{Peak area of } ^{13}\text{C-NMR spectrum 110-160 ppm}}{\text{Total peak area of } ^{13}\text{C-NMR spectrum 0-230 ppm}}$

[¶] IHSS = International Humic Substances Society

The static headspace system used in this study consisted of a glass vial (Supelco, Inc., Bellefonte, PA) fitted with a Teflon screw-cap mininert valve (Alltech Associates, Inc., Deerfield, IL). The total volume of the vials was measured to be 24.85 ml and the liquid volume was 20.00 ml, leaving a headspace volume of 4.85 ml.

To prepare samples for headspace gas chromatography (HSGC) analysis, 20.00 ml of HA or FA solutions (DOC ranged from 0.0 to 4.0 m*M*) in 0.01 *M* KCl, pH=7.0 was added to each vial. Small aliquots (23 - 25 μl) of the herbicide stock solutions were added to each vial, and the vials were immediately capped and allowed to equilibrate in a water bath at 25° C ($\pm$ 0.5° C) for 12 h after thorough mixing. To obtain the Henry's law constants for triallate and trifluralin in 0.01 *M* KCl, pH = 7.0, the procedure was repeated as described above over a range of herbicide concentrations in the absence of HA or FA. In all experiments, the concentrations of these compounds were well within their reported water solubilities. Three replicates were used for each treatment, and standard curves were used to determine the concentration of herbicide in the headspace.

A Varian 3700 gas chromatography (GC) equipped with a ^{63}Ni electron capture detector (ECD) was used to determine the herbicide concentrations in the headspace. The GC column was a wide bore (0.53 mm), 15 m long capillary fused silica column (RSL-200, Alltech Associates, Inc., Deerfield, IL). The carrier gas was a 90/10 mixture of argon and methane with a flow rate of 7 ml/min. and make-up gas flow rate of 23 ml/min. In all experiments, 5 μl of gaseous sample from the headspace or 1 μl of liquid standard was injected into the GC per sample. All GC analyses were carried out isothermally, and the temperature settings of the column, injector and detector for

dichlobenil, triallate and trifluralin are given in Table 8. The syringe was cleaned between injections by washing with methanol and heating.

The influence of ionic strength on the complexation of these herbicides with HA was determined at pH 7 over a range in ionic strengths from 0.001 to 0.100 *M* (0.500 for triallate) adjusted with KCl. The above procedure was repeated to determine the herbicide concentration in the headspace using three replicates per treatment. Additional experiments were also completed to test the interactions among the three herbicides in the presence and absence of HA. A stock solution of mixed herbicides was introduced into the reaction vessels containing different concentrations (0 to 4 m*M* C) of HA in 0.01 *M* KCl, pH = 7.0. The initial concentrations of dichlobenil, triallate and trifluralin in the aqueous solutions were 2.5, 0.5 and 0.5 μM, respectively. After equilibration in the sealed vessels, headspace GC analysis was performed as described previously. The temperature settings for isothermal separation of these three compounds are listed in Table 8. Standard curves of each herbicide were obtained with mixed herbicide solutions to simulate the experimental conditions where all three herbicides were present simultaneously.

Assuming that the primary reaction between the herbicide and HA in solution is complexation (binding), an expression can be derived which allows for the simultaneous estimation of the conditional complexation stability constant and the Henry's law constant. For the complexation reaction

$$\text{Herbicide} + \text{HA} = \text{Herbicide-HA} \qquad [1]$$

the conditional equilibrium constant K (complexation stability constant) is

Table 8. Isothermal gas chromatography analyses of dichlobenil, triallate and trifluralin

Herbicide	Injection Port Temperature (° C)	Column Temperature (° C)	Detector Temperature (° C)	Retention Time (min.)
Dichlobenil	260	150	300	2.8
Triallate	220	190	280	4.5
Trifluralin	260	170	280	5.0
All Three Above	260	165	300	1.9(dich.), 11.7(tria.), 6.5(trif.)

$$K = ([\text{Herbicide-HA}])/([\text{Herbicide}][\text{HA}]) = X/([\text{HA}]C_w) \quad [2]$$

where X and C_w are the concentration of the complex and the free herbicide concentration at equilibrium, respectively. At equilibrium, the original mass of herbicide added can be partitioned as follows:

$$C_oV_w = C_aV_a + C_wV_w + XV_w \quad [3]$$

where C_o is the initial herbicide concentration, C_a is the herbicide concentration in the headspace, and V_a and V_w are the headspace volume and liquid volume, respectively. Solving for X results in

$$X = C_o - C_w - C_a(V_a/V_w) \quad [4]$$

Substitution of Eq. [4] into Eq. [2] with rearrangement results in

$$C_w(K[\text{HA}] + 1) = C_o - C_a(V_a/V_w) \quad [5]$$

At a given temperature, Henry's law states that

$$K_h = C_a/C_w \quad [6]$$

where K_h is the Henry's law constant (unitless when C_a and C_w are expressed as *M*), C_a and C_w are the equilibrium gaseous (headspace) and liquid (water) concentrations of the volatile solute, respectively. Combining Eq. [5] and Eq. [6] with rearrangement leads to:

$$C_o/C_a = (K/K_h)[\text{HA}] + 1/K_h + V_a/V_w \quad [7]$$

A linear relationship between C_o/C_a and the concentration of HA will yield a slope of K/K_h and an intercept of $(1/K_h + V_a/V_w)$. Since V_a/V_w is a constant equal to 0.24 in the present study, the intercept and slope can be used to calculate K_h and K. To calculate the percent of initial herbicide which is complexed with HA or FA, combination of Eq. [2] and Eq. [3] leads to

$$K = X/([HA](C_o - X - C_a(V_a/V_w))) \quad [8]$$

Rearranging Eq. [8] results in:

$$(X/C_o)\ (\%) = 100 \times K[HA](1-(C_aV_a)/C_oV_w)/(1+K[HA]) \quad [9]$$

and if the quantity $(C_aV_a)/C_oV_w)$ is much smaller than 1, then Eq. [9] becomes

$$(X/C_o)\ (\%) \approx 100 \times K[HA]/(1+K[HA]) \quad [10]$$

Results and Discussion

The gaseous concentrations of triallate and trifluralin in the headspace increased as the equilibrium aqueous concentrations of these two compounds increased (Fig. 16). The excellent linear relationship indicates obedience of Henry's law (Eq. [6]). Experiments to determine the K_h for dichlobenil in 0.01 *M* KCl were not performed since there was good agreement on K_h values determined in the presence of HA. The very small negative y-intercepts resulting from curve-fitting are negligible since they were not statistically different (at the 1 % level) than zero. From the slopes, Henry's law constants for triallate and trifluralin in 0.01 *M* KCl at 25° C were 0.20 and 0.48, respectively. Using water solubilities and liquid vapor pressures, and assuming essentially no mutual miscibility between the organic solutes and water, Suntio et al. (1988) estimated the Henry's law constants for dichlobenil, triallate and trifluralin at 20° C to be 0.00028, 0.00042 and 0.0016, respectively. Since the initial concentrations of these herbicides in our experiments were well within the reported water solubilities of these compounds, the disagreement between the measured and predicted Henry's law constants for these

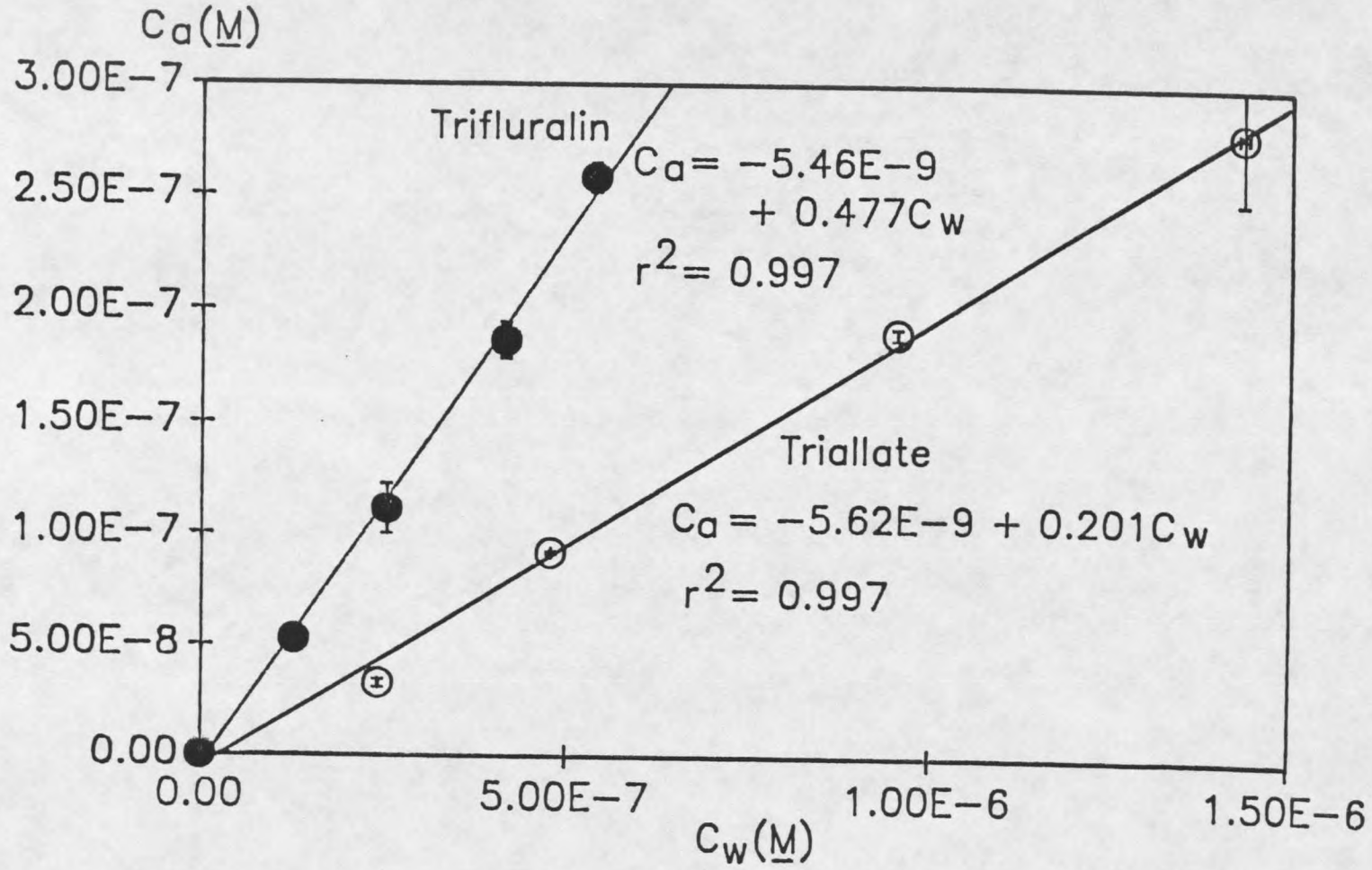


Fig. 16. Headspace gaseous concentration (C_a) vs. aqueous concentration (C_w) plots for triallate and trifluralin. The slopes of the fitted equations are Henry's law constants at 25° C in 0.01 *M* KCl.

herbicides was probably due to the incorrect assumption of no mutual miscibility. This illustrates the importance of experimental determination of K_h and the heavy reliance of assumptions in theoretical predictions.

Plots of C_o/C_a vs the concentration of HA and FA were linear for dichlobenil, triallate and trifluralin (Figures 17-19). The linear decrease in C_a with increasing concentration of HA or FA indicates complexation of these solutes with HA and FA. Eq. [7] was used to calculate K_h and K from the y-intercept and the slope, respectively. Results of linear regression analyses for these three compounds with six HA and FA are summarized in Table 9.

One consistent trend of the complexation of dichlobenil, triallate and trifluralin by dissolved HA and FA was that each solute generally complexed more with HA than with FA, as evidenced by larger K values in the presence of HA (Table 9). This observation is consistent with the study on 1-naphthol (Chapter 2) and other studies on DDT and polycyclic aromatic hydrocarbons (Carter and Suffet, 1982; Gauthier et al., 1986) and can be explained in terms of hydrophobic partitioning (Chiou et al., 1983). HA usually contains more hydrophobic character than FA and thus provides more hydrophobic regions for partitioning of hydrophobic solutes such as dichlobenil, triallate and trifluralin.

The Henry's law constants, determined using linear regression of C_o/C_a vs. HA or FA concentrations plots (Table 9), were very consistent with those obtained from the C_a vs. C_w plots (Fig. 16). For example, the 95 % confident interval for the Henry's law

Table 9. Henry's law constants (K_h, unitless) and conditional complexation constants (K) calculated from the y-intercepts and slopes of C_o/C_a vs HA or FA concentration plots (Eq. [7]).

Humic Substances	Dichlobenil			Triallate			Trifluralin		
	K_h	K (L/g C)	r^2	K_h	K (L/g C)	r^2	K_h	K (L/g C)	r^2
IHSS HA 1R102H	0.0096	10.5	0.974	0.19	30.2	0.998	0.52	19.1	0.987
IHSS HA 1R106H	0.0094	8.9	0.987	0.21	39.0	0.990	0.54	19.8	0.987
Aldrich HA	0.0095	8.6	0.944	0.20	45.6	0.976	0.56	26.2	0.953
MT Soil HA	--	--	--	0.19	45.7	0.949	--	--	--
IHSS FA 1S102F	--	--	--	--	--	--	0.52	10.1	0.994
Wheat-straw FA	0.0096	5.9	0.991	0.21	17.2	0.964	0.52	10.8	0.998

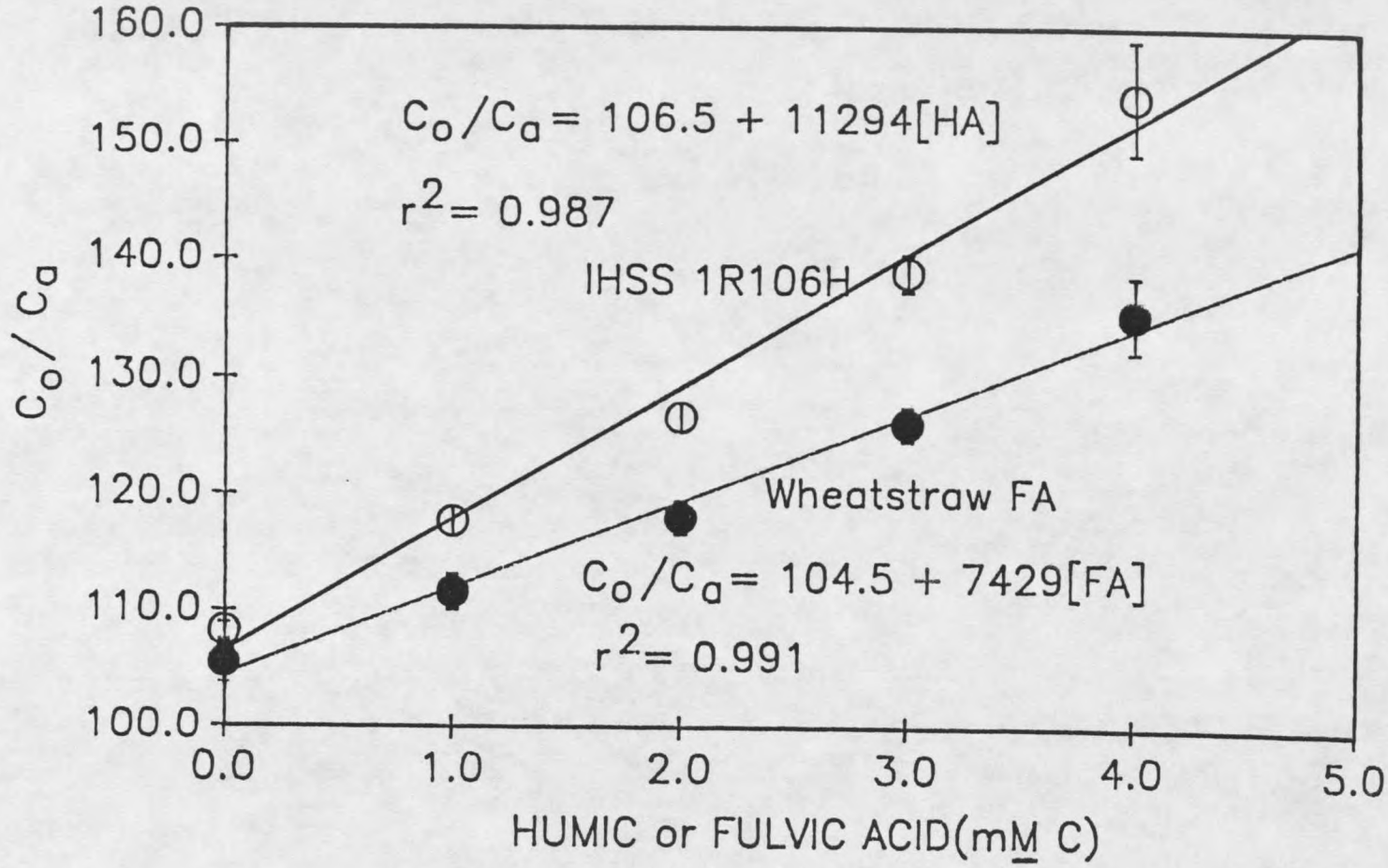


Fig. 17. Complexation of dichlobenil by HA and FA. IHSS = International Humic Substance Society. Initial dichlobenil concentration $C_o = 5 \times 10^{-6}$ *M*.

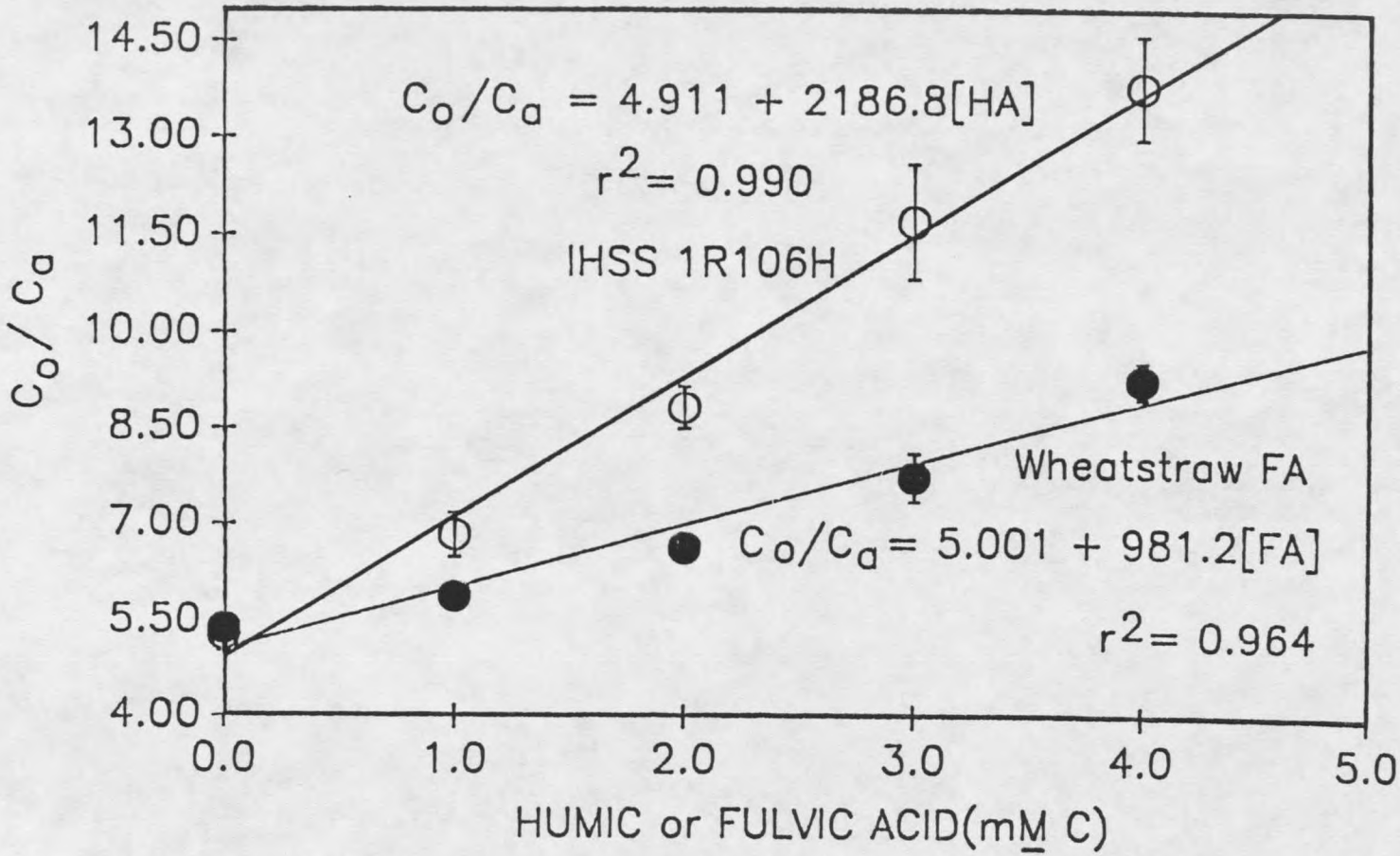


Fig. 18. Complexation of triallate by HA and FA. IHSS = International Humic Substance Society. Initial triallate concentration $C_o = 5 \times 10^{-7}$ *M*.

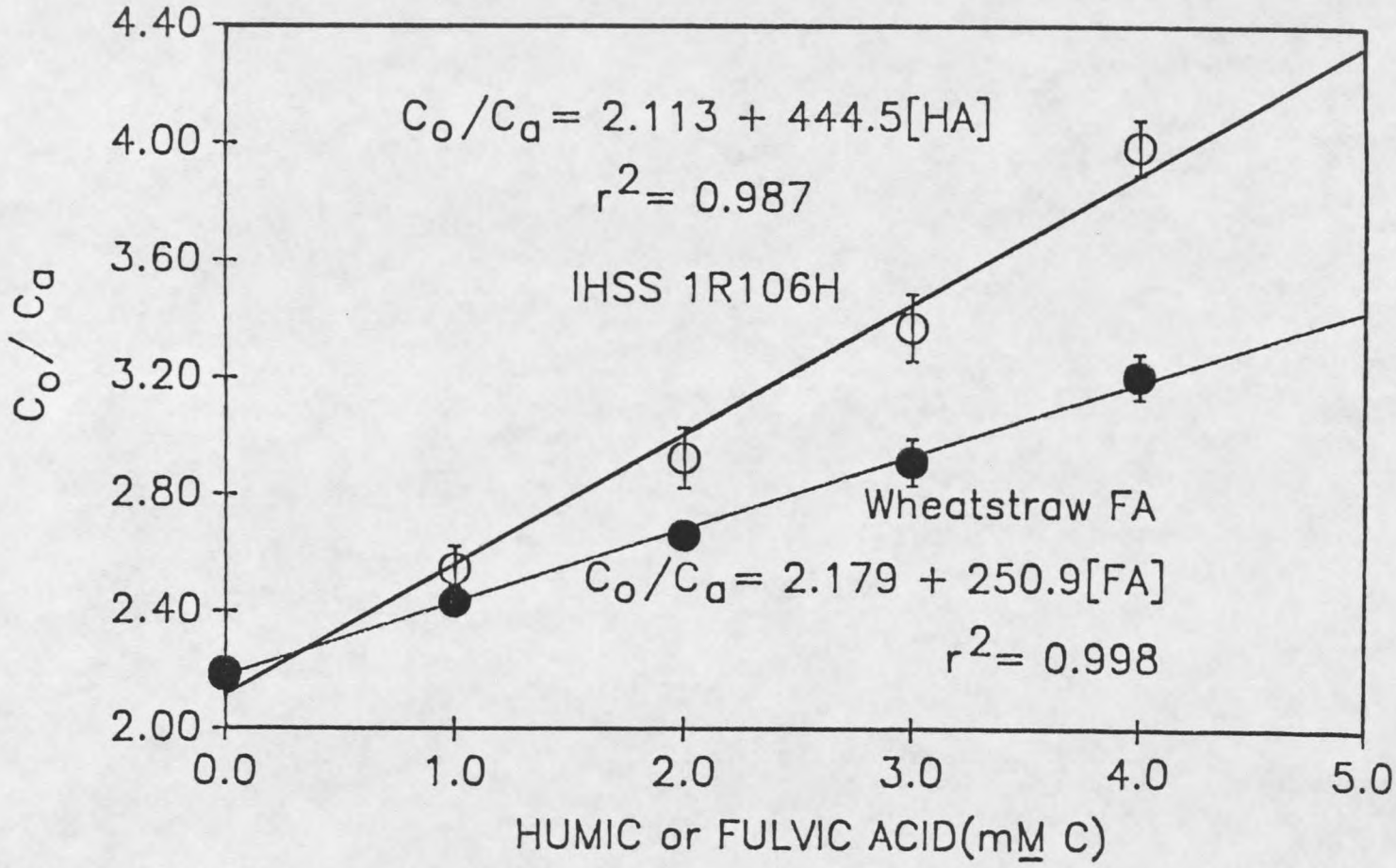
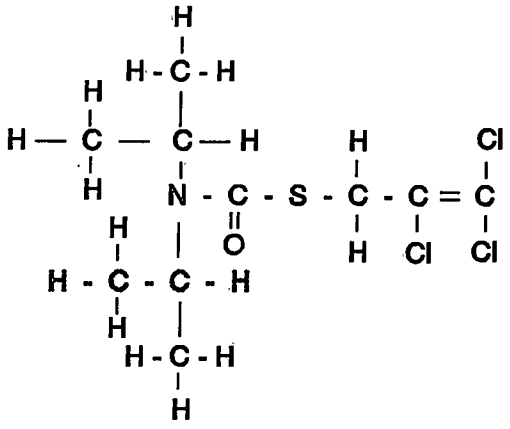


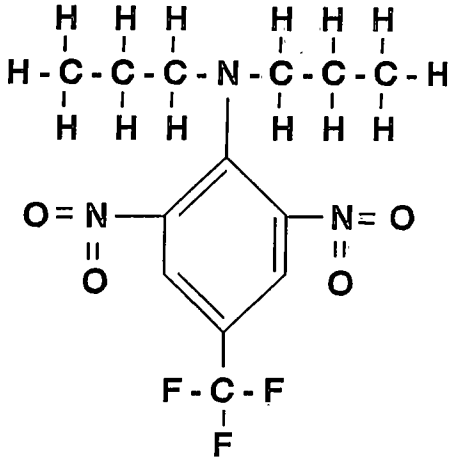
Fig. 19. Complexation of trifluralin by HA and FA. IHSS = International Humic Substance Society. Initial trifluralin concentration $C_o = 5 \times 10^{-7}$ *M*.

constant for trifluralin was (0.45, 0.51), compared with values ranging from 0.52 to 0.56 obtained from the C_o/C_a vs. HA or FA concentrations plots. K_h values for triallate ranged from 0.19 to 0.21, very close to the value of 0.20 obtained from the C_a vs. C_w plot. The average K_h for dichlobenil was 0.0095, and all values were very consistent.

Using water solubility as the criterion to rank the hydrophobicity of these three compounds, the hydrophobicity should increase in the order: trifluralin > triallate > dichlobenil. Since the major mechanism for the complexation of organic solutes by dissolved humic substances is hydrophobic association (Chiou et al., 1983), then we would expect that the strength of complexation should follow the order of hydrophobicity for these compounds. However, experimental data showed that the complexation constants increased in the order: triallate > trifluralin > dichlobenil. Therefore, other factors besides hydrophobicity have to be considered in explaining the discrepancy between triallate and trifluralin. One potential explanation is the difference in chemical structures. Triallate is a chain-shape, nonaromatic molecule while trifluralin is a plane-shape aromatic molecule containing 3 functional groups and a side chain. These molecules are similar with the exception of the substitution on the amine N: triallate has a thiocarbonyl group while trifluralin has a nitro and trifluoromethyl substituted aromatic ring. Hence, it is reasonable to propose that the difference in the strength of complexation may lie in the difference in the thiocarbonyl and substituted aromatic ring structures. Evidently, a linear structure is preferred over an aromatic ring shielded by electron withdrawing functional groups. However, it is not known if the electron-withdrawing nitro and trifluoromethyl groups played a role in the complexation reaction.



Triallate



Trifluralin

Changes in ionic strength ranging from 0.001 to 0.100 (0.500 for triallate) *M* resulted in no statistical differences in C_o/C_a ratios (Eq. [7]) in the presence of 2 m*M* C HA for all three herbicides (Figures 20-22). At a fixed HA concentration, the C_o/C_a ratio is a function of only K/K_h and $1/K_h$ (Eq.[7]). Consequently, no significant change in C_o/C_a with changes in ionic strength indicates that either (i) K and K_h both changed with ionic strength resulting in no net change in C_o/C_a, or (ii) neither K or K_h changed significantly with changes in ionic strength. Other studies have shown that conditional complexation constants for nonionic organic solutes complexed by dissolved humic substances are not sensitive to solution ionic strength (Chen et al., 1992; Gauthier et al., 1986; Traina et al., 1989). Also, Gossett (1987) reported that ionic strength must reach rather substantial values (> 0.2 *M* KCl) to cause a 10% increase in the apparent Henry's law constant for tetrachloroethylene. Consequently, from the data presented here and observations of other studies, we concluded that the Henry's law constants and the

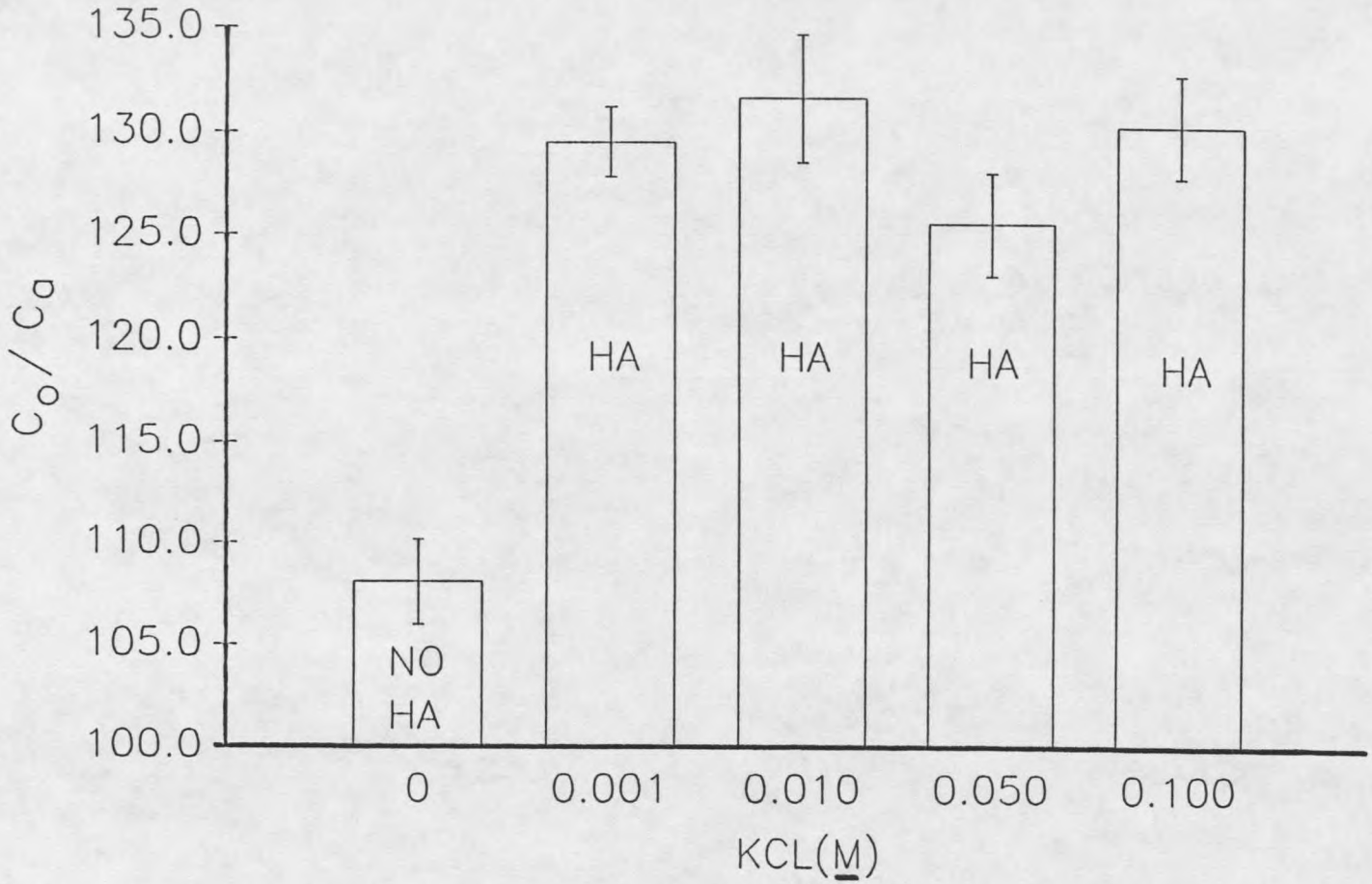


Fig. 20. Effects of ionic strength (KCl concentration) on the complexation of dichlobenil by a HA (IHSS IR106H) and on the Henry's law constant.

Fig. 21. Effects of ionic strength (KCl concentration) on the complexation of triallate by a HA (IHSS IR106H) and on the Henry's law constant.

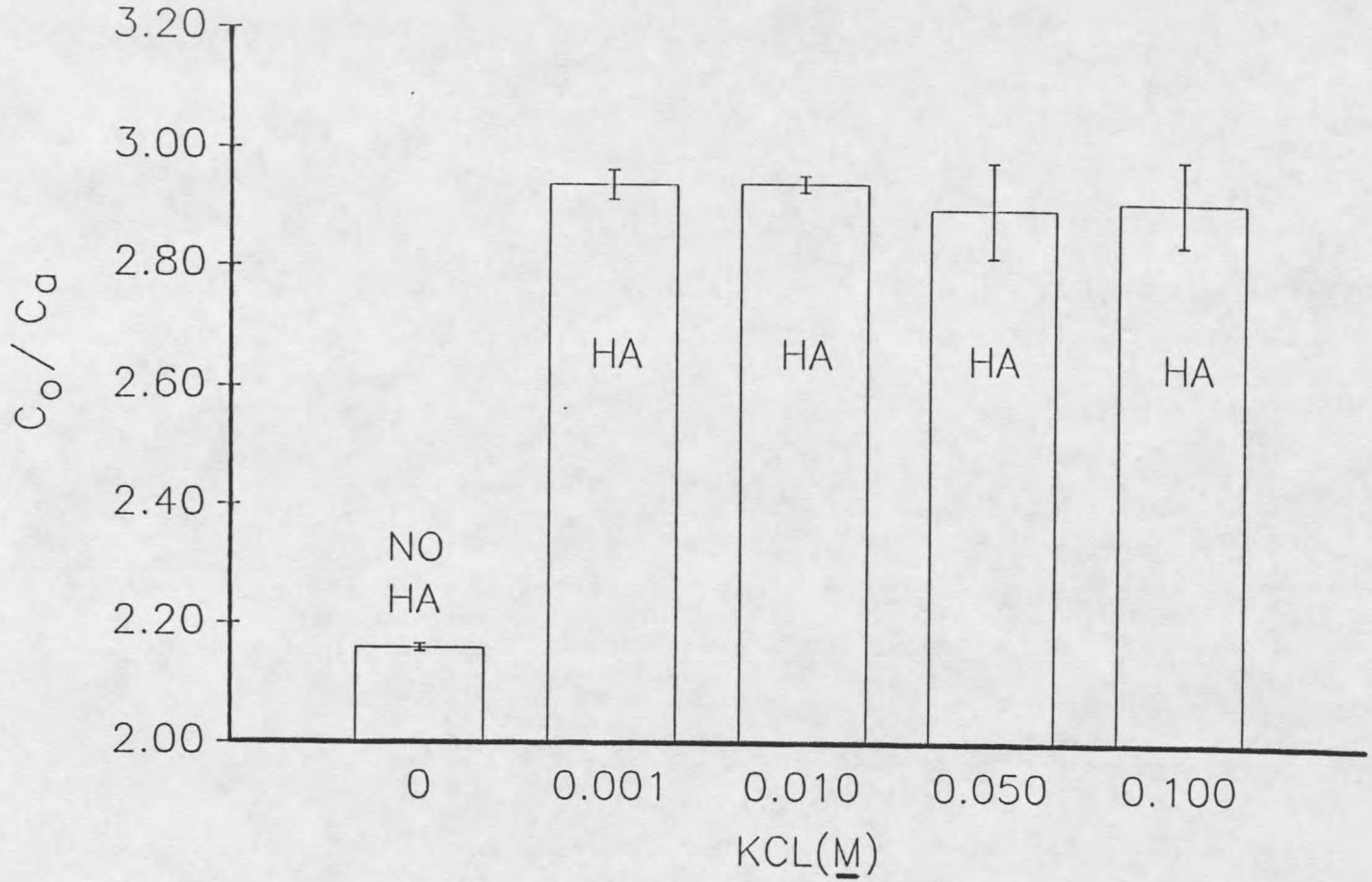


Fig. 22. Effects of ionic strength (KCl concentration) on the complexation of trifluralin by a HA (IHSS 1R106H) and on the Henry's law constant.

complexation constants with HA for dichlobenil, triallate and trifluralin were not sensitive to the solution ionic strength.

When dichlobenil, triallate and trifluralin were present simultaneously, the Henry's law constants and the conditional complexation constants were not significantly different ($\alpha = 1$ %) than those obtained for each compound singularly (Table 10). If the complexation of these herbicides by dissolved HA was primarily partitioning or association with hydrophobic regions of the HA, then one would expect that competition for hydrophobic sites would lead to redistribution of association sites with different solutes and thus potentially different complexation constants for each herbicide. The fact that we did not observe changes in complexation constants for the three herbicides when they were present simultaneously suggests that at the herbicide and HA concentrations used, we did not exceed the capacity of HA sites available for complexation. However, this simple assumption can be complicated by solute-solute-humic acid interactions. Alternatively, if these solutes exhibit preferences for different sites on the HA molecule, then displacement due to site competition may be unlikely. Thus, it is important to note that complicated interactions may have occurred among solutes and HA in the solution even though the complexation constants remained unchanged. Under natural conditions (e.g. soil solutions, surface waters) where the concentration of DOC may often exceed the concentration of contaminants by several orders of magnitude, it appears that the presence of several solutes at low concentration will not inhibit solute-humic acid interactions.

Table 10. Comparison of Henry's law constants and conditional complexation constants (K) with IHSS 1R102H HA obtained when dichlobenil, triallate and trifluralin were present singularly and simultaneously.

Herbicide	K		K_h		r^2
	Singular	Mixed	Singular	Mixed	Mixed
Dichlobenil	10.5	10.6	0.0096	0.0096	0.991
Triallate	30.2	29.7	0.19	0.19	0.999
Trifluralin	19.1	22.2	0.52	0.53	0.990

The Henry's law constant of each solute, obtained in the presence of the three herbicides, did not change significantly as compared with those obtained singularly. With solution concentrations of the herbicides in the range of 0.5 to 2.5 μM, the presence of 2 or more herbicides was insignificant as compared with the 0.01 M KCl already in solution. As discussed earlier, Henry's law constants are relatively insensitive to the solution ionic strength. In addition, the concentrations of herbicides in the gas phase were approximately 10^{-8} M, consequently, we would expect very little interference among the gaseous herbicide molecules.

Generally, complexation of these solutes by dissolved humic substances can reduce the vapor pressure of these herbicides. For example, with IHSS 1R102H HA, it was estimated that 20.1%, 40.9% and 28.9% of the added dichlobenil, triallate and

trifluralin, respectively, formed water soluble complexes with 2 m*M* C of HA in the solution (Eq. [9], Fig. 23). Since complexation of the herbicides with HA greatly reduced their free concentrations in the aqueous solution, according to Henry's law (Eq. [6]), the gaseous herbicide concentrations at a given temperature should also decrease, and thus volatilization is reduced.

Conclusions

The Henry's law constants and conditional complexation constants for dichlobenil, triallate and trifluralin binding to HA in aqueous solutions were estimated simultaneously using the relationship between C_o/C_a and HA or FA concentration. The magnitude of the Henry's law constants for these compounds followed the order of hydrophobicity (water insolubility) of these compounds: trifluralin > triallate > dichlobenil. Among these compounds, triallate was preferentially complexed by dissolved HA, although trifluralin is slightly more hydrophobic than triallate. The conditional complexation constants for all of the humic substances used in this study increased in the order: triallate > trifluralin > dichlobenil. Among the humic substances used in this study, humic acids showed a stronger tendency to form complexes with these compounds than fulvic acids because of their more abundant hydrophobic sites.

The ionic strength of the solution, or the simultaneous presence of low concentrations of these compounds in the solution did not have significant effects on the Henry's law constants and the conditional complexation constants.

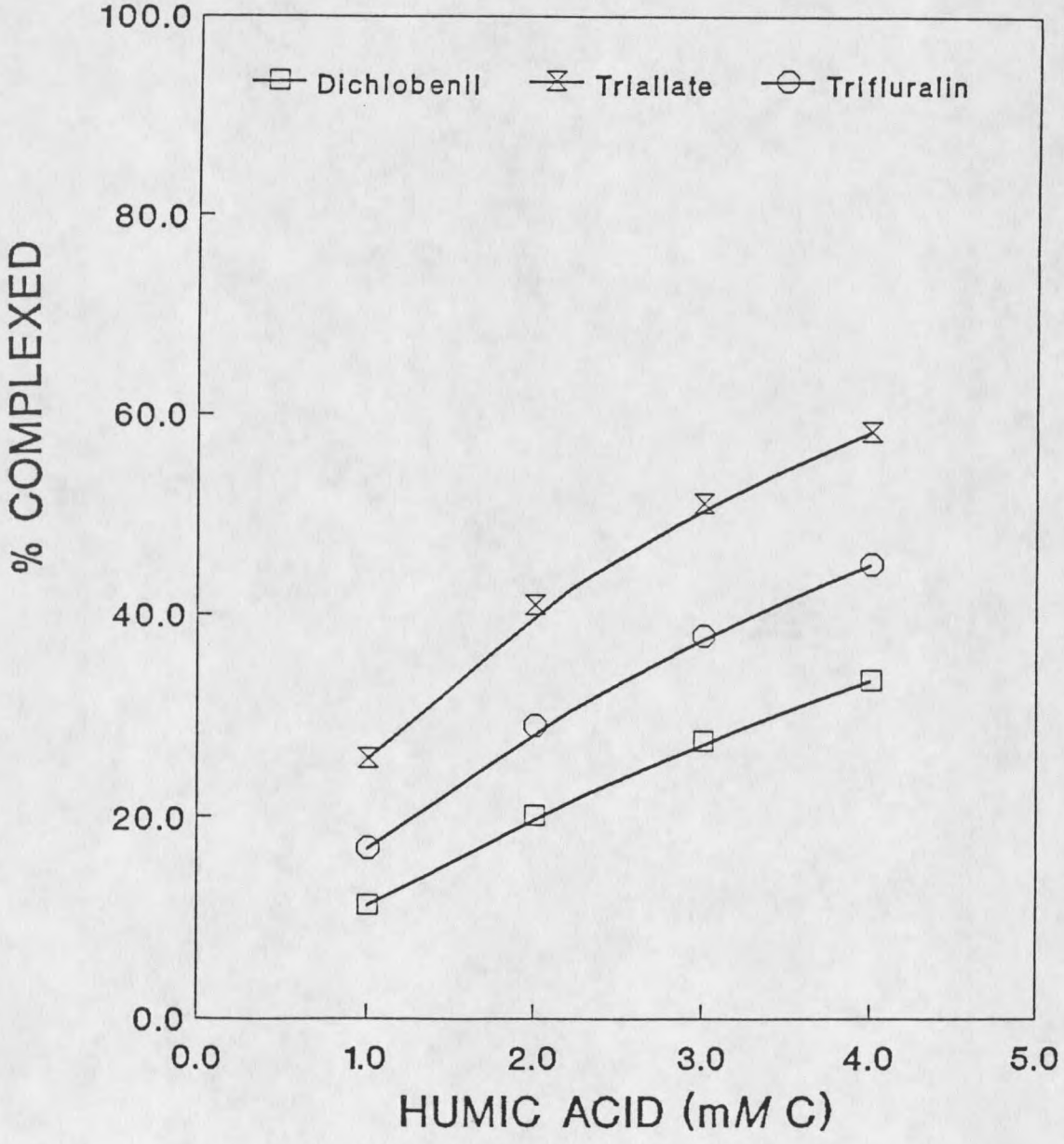


Fig. 23. Percentage of added dichlobenil, triallate and trifluralin forming water soluble complexes with dissolved HA (IHSS reference soil humic acid 1R102H).

Using the complexation constants determined in this study, approximately 20, 41 and 29 percent of added dichlobenil, triallate and trifluralin formed water soluble complexes with 3 m*M* C as HA. Consequently, complexation of these herbicides with dissolved humic substances is an important process in determining the behavior and fate of these chemicals in natural systems.

CHAPTER 5

EFFECTS OF DISSOLVED HUMIC ACIDS ON THE PHYTOTOXICITY OF ATRAZINE, PICLORAM AND TRIALLATE

Introduction

Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine), picloram (4-amino-3,5,6-trichloropicolinic acid) and triallate (s-(2,3,3-trichloroallyl) diisopropylthiocarbamate) are widely used soil applied herbicides. Atrazine is a selective herbicide for control of broadleaf and grassy weeds in agricultural production, and picloram is used for general woody plant control of most annual and perennial broad leaf weeds. Picloram is heavily used in Montana to control spotted knapweeds and leafy spurge. Triallate is a popular herbicide for control of wild oats in barley and wheat in Montana.

The efficacy of soil applied herbicides can be influenced by a number of factors, but one of the most significant factors is soil organic matter (Blumhorst et al., 1990; Schmidt and Pestemer, 1980; Walker, 1980). Capriel et al. (1985) showed that substantial amounts of atrazine and its metabolites became bound to humic substances nine years after herbicide application. Other studies have also shown that picloram can be adsorbed strongly by soil humic acids (Nearpass, 1976; Khan, 1973). Humic substances, especially humic (HA) and fulvic (FA) acids, represent a significant fraction of organic matter in soils, which may ultimately affect herbicide adsorption to soils, herbicide absorption by plants, and subsequent efficacy of the herbicide (Schmidt and Pestemer, 1980). The presence of dissolved humic substances has been found to reduce

atrazine inhibition of sulfate uptake by excised barley roots (Dell'Agnola et al., 1981), and bioavailability of organic contaminants to an amphipod in interstitial waters (Landrum et al., 1987). In addition, a number of studies have shown that nonionic organic solutes, including many pesticides, will form water soluble complexes with humic and fulvic acids (Chiou et al., 1983, 1986; Carter and Suffet, 1982, 1983). Previous chapters (2, 3 and 4) have also shown the formation of soluble complexes of 1-naphthol, dichlobenil, triallate and trifluralin with dissolved humic substances. However, very little information exists on whether soluble pesticide-HA complexes influence pesticide bioavailability or pesticide efficacy.

The objectives of this study were to (i) determine if HA reduces the phytotoxicity of atrazine, picloram and triallate using bioassay techniques, (ii) measure the effects of herbicidal exposure time on the phytotoxicity of atrazine in the presence and absence of HA, and (iii) test if there is a relationship between the strength of herbicide-dissolved HA interaction (complexation) and the reduction in bioavailability.

Materials and Methods

The bioassay indicator species used in this study were oats (*Avena sativa* L.) for atrazine and triallate, and tomato (*Lycopersicom esculentum*) for picloram. Seedlings of the indicator plants were grown in sand culture before they were transferred to a hydroponic culture medium. The hydroponic culture medium was a 1:100 dilution of Hoagland's solution with minor modification (Hoagland and Arnon, 1950; Langhans,

1978). A modified nutrient solution (100-times less Ca and Mg, pH adjusted to 6.0) was used when HA treatments were applied to avoid flocculation of HA.

A commercial HA (K & K Laboratories, Inc., Plainview, NY) was used in all experiments with atrazine. Another commercial HA (Aldrich Chemical Co., Milwaukee, WI) was used for atrazine, picloram and triallate. The Aldrich HA was purified using the procedures recommended by Thurman and Malcolm (1981) prior to use in the nutrient solutions. Stock HA solutions were prepared by dissolving the solid HA with dilute KOH (pH $\approx$ 8 - 9), adjusting the pH to 7.0, then filtering through 0.45-μm filters. The HA nutrient solutions were prepared by mixing the modified nutrient solution with the HA stock solution and adjusting the pH to 6.0. The total dissolved organic carbon (DOC) of the HA nutrient solutions was determined with a Dohrmann DC-80 C analyzer (Dohrmann, Santa Clara, CA).

Stock solutions of the herbicides were prepared by dissolving analytical grade atrazine (98.7 % pure, Ciba-Geigy Corp., Greensboro, NC), picloram (99.4 % pure, DowElanco, Midland, MI) and triallate (99.9 % pure, Monsanto Co., St. Louis, MO) into spectrum-grade methanol (EM Science, Cherry Hill, NJ).

The container of the nutrient solutions was a 1-liter size non-transparent plastic bottle wrapped with white paper to prevent heating effects of sunlight. There were 3 openings in the cap: two to accept plants and one for aeration. Each nutrient solution vessel was aerated daily with an air pump.

All experiments were conducted in a greenhouse with an air temperature of 21 °C ($\pm$ 5 °C) day-time and 13 °C ($\pm$ 3 °C) night-time. Six hours of supplemental lighting

was provided daily. The photon flux density (400-700 nm) in the greenhouse ranged from 110 to 800 μmol m^{-2} s^{-1}.

Aliquots of the herbicide stock solutions were added to the nutrient solutions receiving the herbicide while the nutrient solution was being agitated and stirred with air. The final concentration of methanol in the herbicide treatment ranged from 0.002 to 0.008 % (v/v), consequently, all non-herbicide treatments also received 0.008 % (v/v) methanol. Each treatment was replicated 5 times. Immediately following the herbicide treatments, all plants were given a recovery period in complete nutrient solution. After the recovery period, the plants were dried with paper towels, measured for plant height, separated into upper parts and roots, and fresh weights obtained.

Atrazine

To test the effects of humic acids (HA) on the phytotoxicity of atrazine, two humic acids (K&K and Aldrich) were used, and the experimental procedures were carried out as described above. Preliminary experiments showed that in the presence of 0.5 μM atrazine, oat plants had to be less than 21 days old to obtain a suitable atrazine bioassay. For each experiment, atrazine (0 - 10 μM) was added to the nutrient solutions containing oats in the 2 - 2.5 leaf stage (about 16 days old) to obtain a standard curve. The recovery period was 3 days. To test the effects of herbicidal exposure time on phytotoxicity in the presence of HA (3.24 mM C), oat plants were exposed to atrazine at 0.5 μM for 2, 4 and 6 days before giving them a recovery period of 3 days. In all these experiments, four oat plants were planted in each vessel (replicate), and all plants were grown hydroponically for at least one week before initiation of treatments.

Picloram

To test the effects of HA on picloram phytotoxicity, tomato plants in the two true leaf stage were exposed to 0.2, 0.6 and 1.0 μM picloram in the presence and absence of 2.72 mM C as HA for 3 days followed by a recovery period of 4 days. A standard curve between tomato fresh weight and picloram concentration ranging from 0 - 1.4 μM was developed utilizing the same procedures in the absence of HA. Two tomato plants were used in each of the five replicates per treatment.

Triallate

To test the effects of HA on triallate phytotoxicity, oat plants in the three leaf stage were exposed to 0.1, 1.0 and 6.0 μM triallate in the presence and absence of 2.7 mM C as HA for 3.5 days followed by a recovery period of 6 days. A standard curve relating oat plant height and triallate concentration was developed over triallate concentrations ranging from 0 to 12.0 μM. Four oat plants were used in each of the five replicates per treatment. Both the height (from the base of the stem to the base of the second true leaves) and the fresh weight of the oat plants were measured immediately after termination of the experiment.

Results and Discussion

Atrazine

Addition of atrazine to nutrient solutions containing oat plants resulted in chlorosis in leaves and reduction in fresh plant weight (Fig. 24). Both the whole plant weight and plant weight excluding roots (upper plant weight) were suitable parameters for atrazine

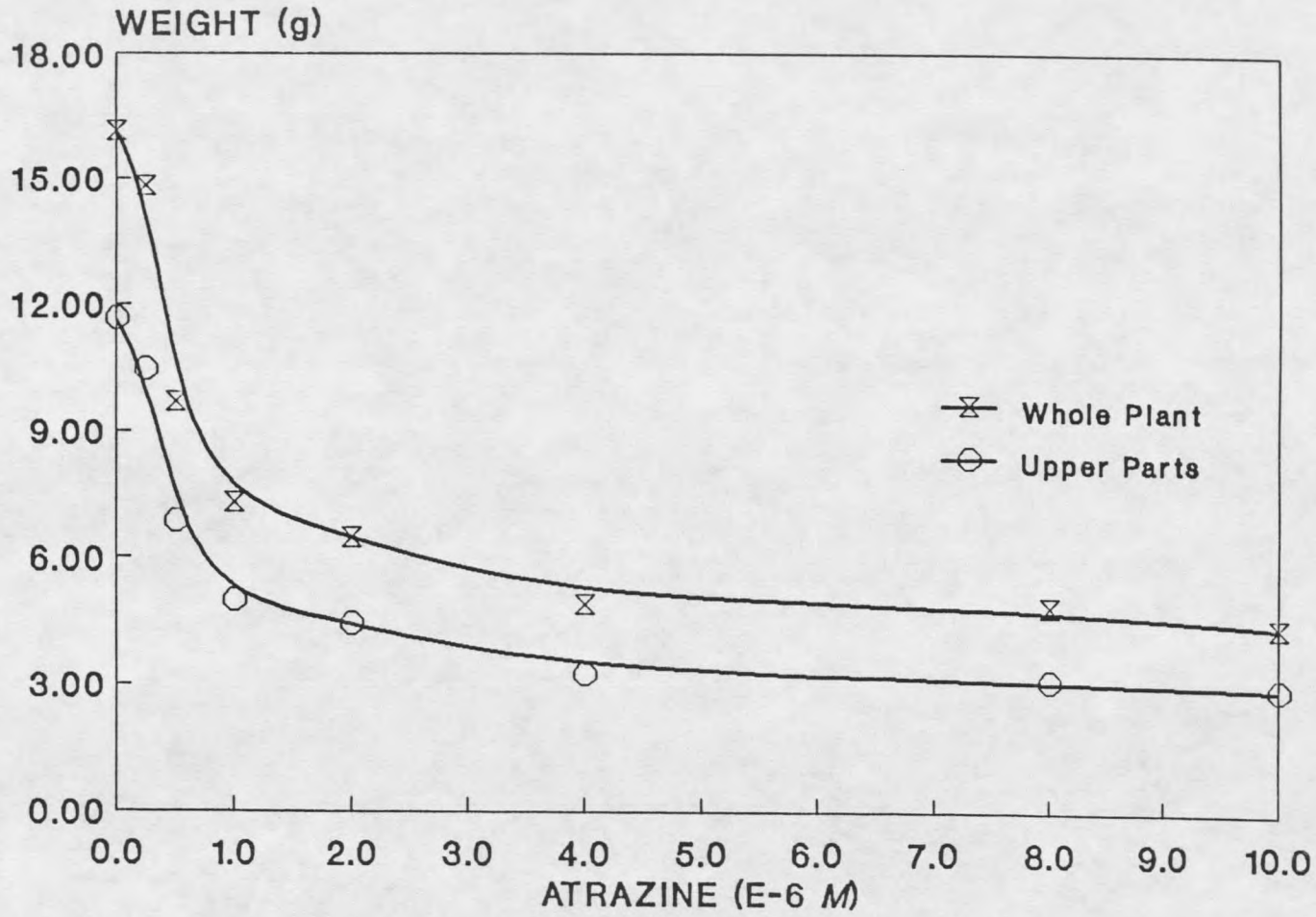


Fig. 24. Standard curve for atrazine bioassay: whole plant fresh wight of oats vs. atrazine concentration. The standard deviation (SD) for the whole plant data ranged from 0.367 to 2.380 and the SD for the upper parts data ranged from 0.282 to 1.758.

bioassay. Although the reduction in fresh weight was not linear over the range of atrazine concentrations (0.0 to 10.0 μM), the sensitivity was much higher compared with other atrazine bioassay methods (Brattain et al., 1983; Shaw et al., 1985). Log-log plots of whole plant weight vs atrazine concentration were linear and resulted in the following regression equations (Eq. [1] for the K&K HA experiment and Eq. [2] for the Aldrich HA experiment):

$$\log (\text{weight}) \approx - 0.909 - 0.304(\log[\text{atrazine}]) \quad [1]$$

$$\log (\text{weight}) \approx - 0.895 - 0.297(\log[\text{atrazine}]) \quad [2]$$

The linear correlation coefficients (r) for Eq. [1] and [2] were - 0.968 and - 0.982, respectively.

Addition of HA in the absence of atrazine did not show significant (at 90 % level) effects on plant growth and fresh weight. However, when HA was added to nutrient solutions containing 1, 2, or 4 μM atrazine, there was a significant reduction (increase in fresh weight) in atrazine toxicity to oats (Fig. 25). In the absence of HA, the fresh weights of oats subjected to the three atrazine concentrations ranged from 30 to 45 % of that of the control, while in the presence of HA, the fresh weights ranged from 44 to 56 % of that of the HA control. The increase in whole plant weight in the presence of HA ranged from 18 to 39 percent compared to atrazine treatments in the absence of HA. Both the Aldrich and the K&K HA exerted similar reductions in phytotoxicity due to atrazine. The similar effects of the Aldrich and the K&K HA on atrazine phytotoxicity were expected since both HA are very similar. Using the whole plant weight of oats grown in the presence of HA and Eq. [1] and [2], it was calculated that the bioavailable

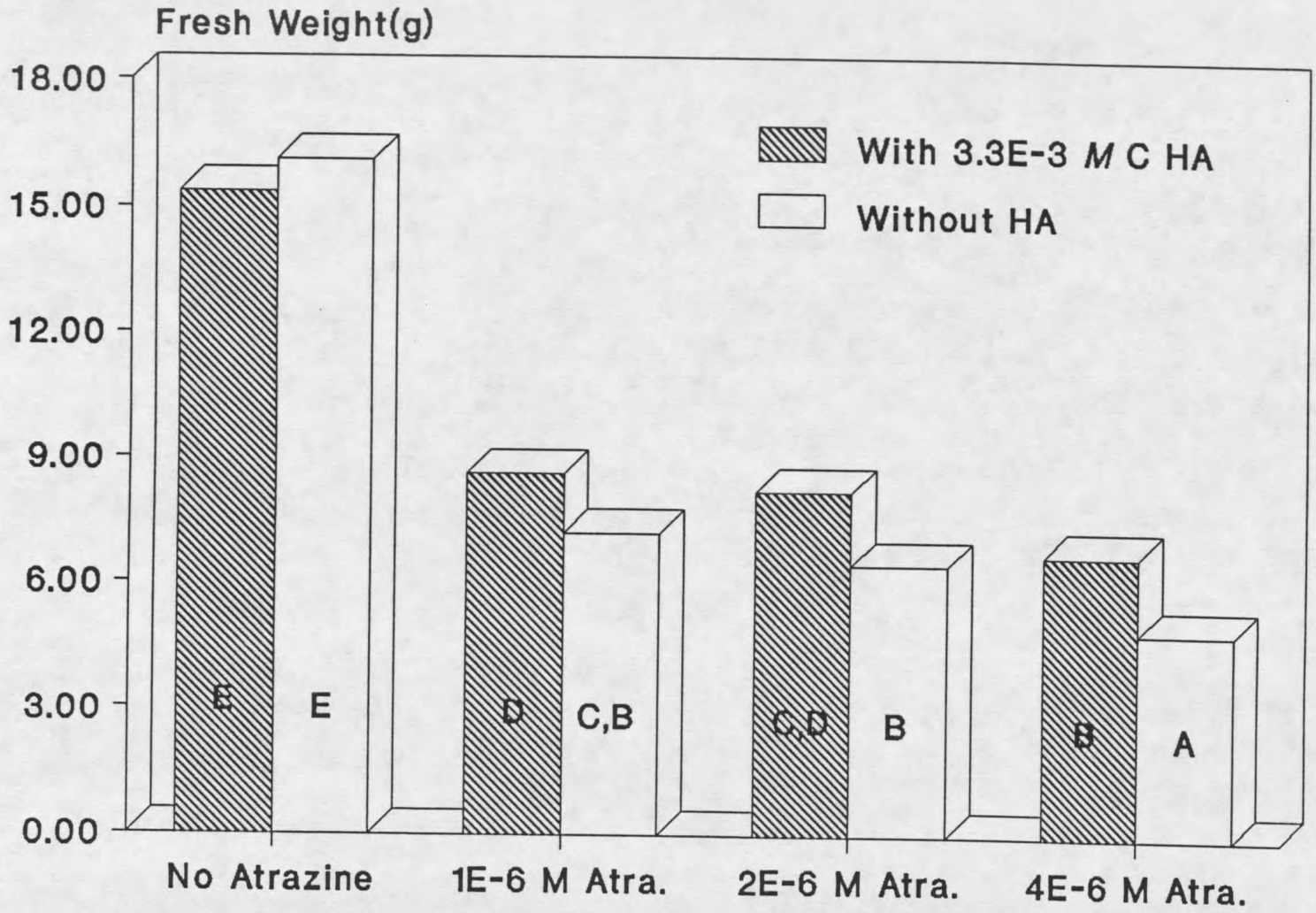


Fig. 25. Effects of K&K HA on the phytotoxicity of atrazine to oats. LSD = 1.147. Different letters indicate 90% significantly different groups. [HA] = 3.26 m*M* C.

concentrations of atrazine were 0.5 - 0.9, 1.1 - 1.4 and 1.8 - 1.9 μM in the 1.0, 2.0 and 4.0 μM atrazine solutions, respectively.

Atrazine Exposure Time

Increasing the atrazine exposure time from 2 to 6 days resulted in significant (at 90% level) reductions of whole plant fresh weight of oats from 11.43 to 7.42 g (Fig. 26). The presence of 3.4 mM C as HA resulted in significant reductions in atrazine toxicity to oats (increases in fresh weight) at all exposure times. Percent increases in whole plant weight as a result of HA were 41, 44 and 35 % for exposure times of 2, 4 and 6 days, respectively. Again, addition of HA reduced the toxicity of atrazine even though the herbicidal exposure times were different. Since atrazine is a photosynthesis inhibitor, and its absorption is believed to be a passive process (Schmidt and Pestemer, 1980), its accumulation in oats is proportional to the length of herbicidal exposure. For passive herbicide uptake, the phytotoxicity is proportional to the bioavailability of the herbicide, and this experiment suggested that the reduction in atrazine phytotoxicity to oats was mainly due to reduction in bioavailable concentrations of atrazine.

Picloram

There was a strong relationship between fresh weight of tomato and picloram concentration (Fig. 27). The range of picloram concentrations was well within the ranges used by other investigators (Lacey, 1986; Reid and Hurt, 1969). A log-log relationship with a linear correlation coefficient (r) of - 0.987,

$$\log (\text{weight}) \approx - 1.804 - 0.374(\log[\text{picloram}]) \qquad [3]$$

adequately described the relationship between plant weight and picloram concentration.

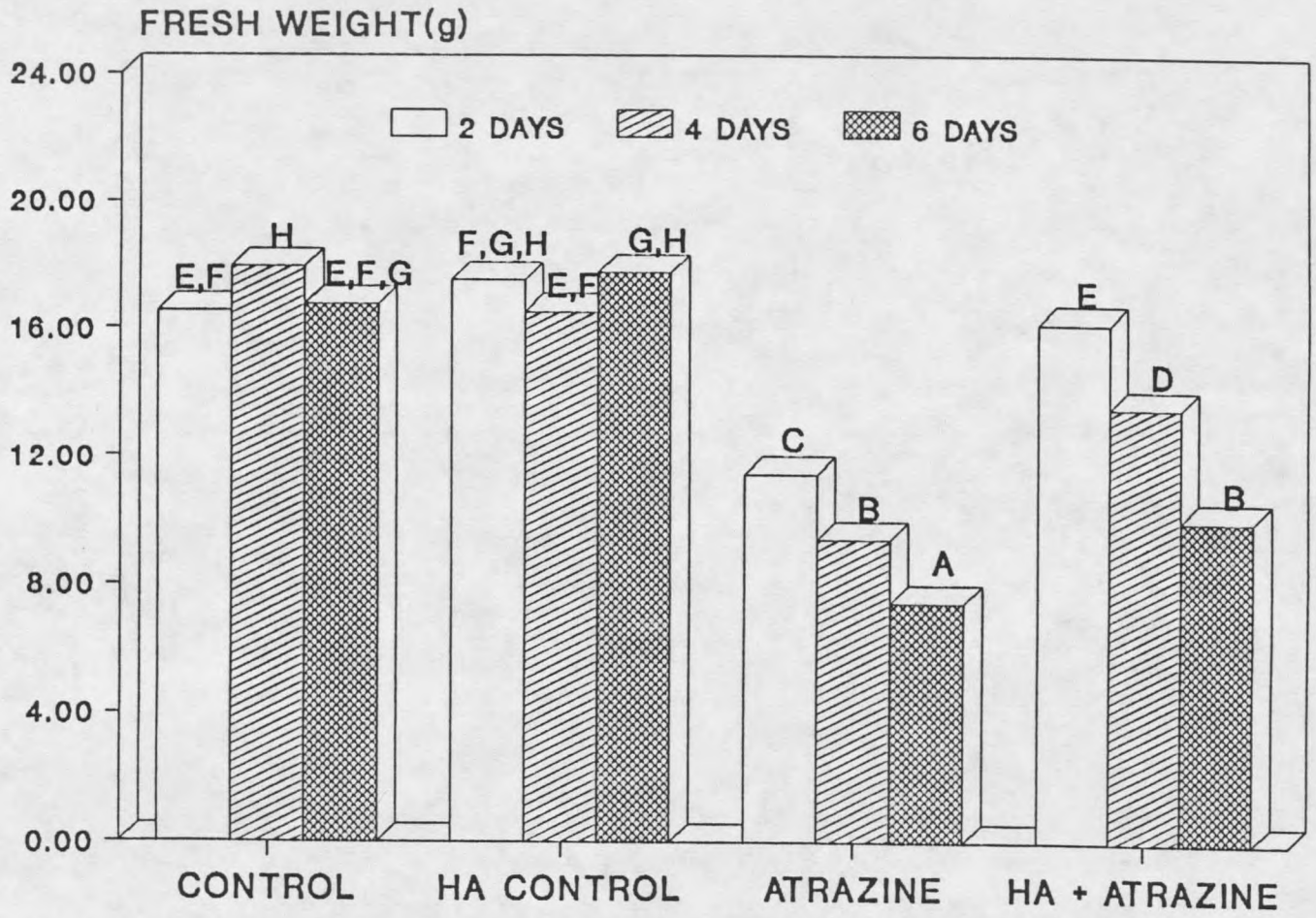


Fig. 26. Effects of atrazine exposure time on the phytotoxicity of 0.5 μM atrazine to oats in the presence and absence of 3.40 mM C Aldrich HA. Oat plants were 4.5, 5.0 and 5.5 leaves old respectively when the treatment started. LSD = 1.187. Different letters indicate significant difference at the 90 % level.

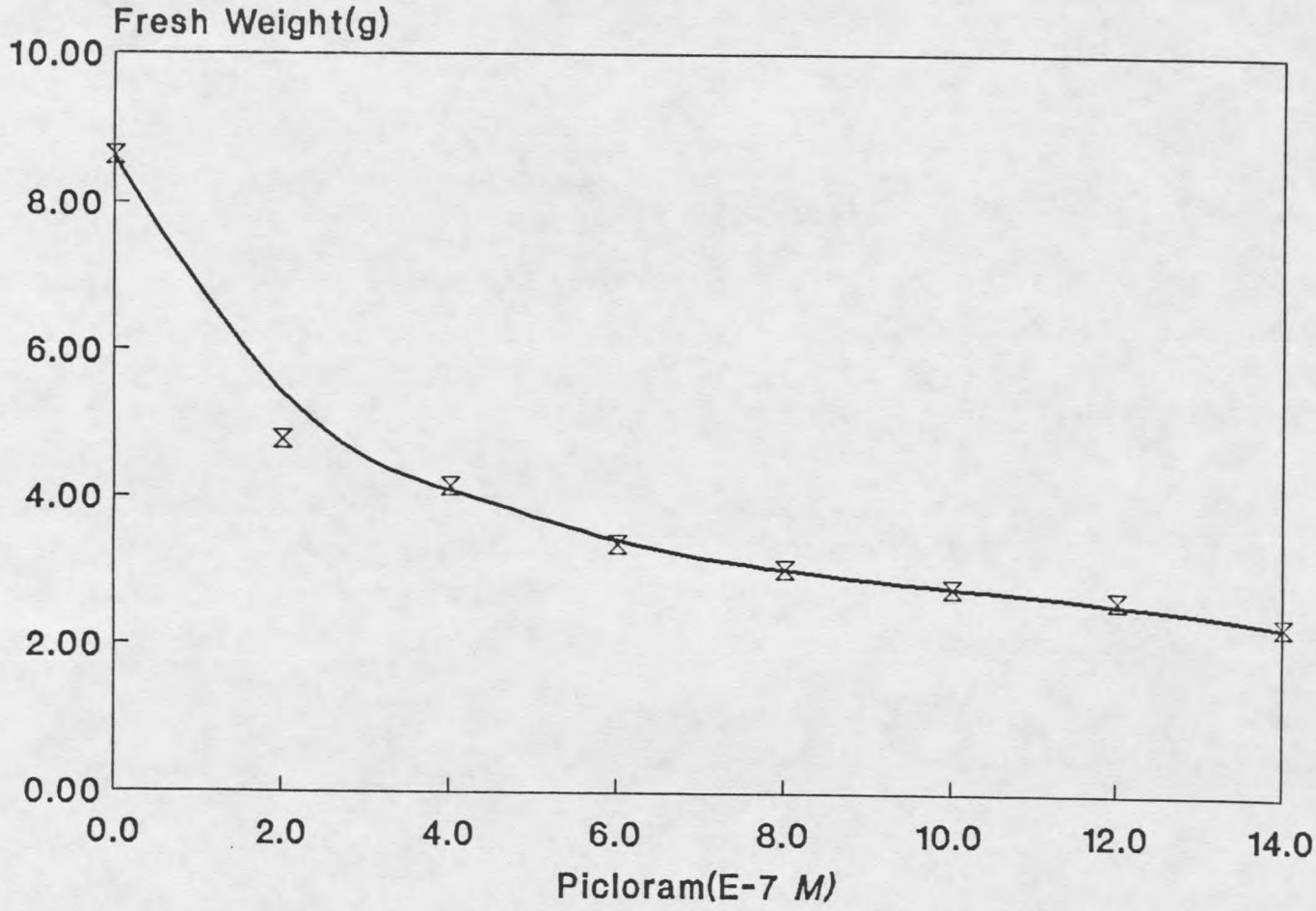


Fig. 27. Standard curve for picloram bioassay: whole plant fresh weight of tomatoes vs. picloram concentration. The standard deviation (SD) for the data ranged from 0.50 to 1.16.

In the absence of picloram, addition of HA (2.7 m*M* C) did not significantly (90 % level) change the fresh weight of tomato plants. However, when HA was introduced to nutrient solutions containing 0.2, 0.6 and 1.0 μM picloram, there were significant reductions in picloram phytotoxicity (increases in plant weight) to tomatoes (Fig. 28). Without HA, the fresh weights were 32 to 55 % of that of the control; while with HA, the corresponding range was 40 to 65 %, and the percent increase in fresh weight ranged from 28 to 38 compared with the picloram treatments in the absence of HA. Using the fresh plant weight of tomatoes grown in the presence of HA and Eq. [3], it was estimated that 0.12, 0.27 and 0.42 μM of picloram was biologically available to the tomato plants when 0.2, 0.6 and 1.0 μM picloram was initially present in the HA nutrient solutions.

Triallate

The fresh weight of oat plants decreased with increasing triallate concentration in nutrient solutions, however, the sensitivity of this plant parameter to triallate was low (data not shown). The plant height (from the base of the stem to the base of the second true leaves) offered better sensitivity over a range of triallate concentrations (0 - 6.0 μM, Fig. 29). A log-log relationship with a correlation coefficient (r) of - 0.993 was established between oat plant height and triallate concentration:

$$\log (\text{height}) \approx - 1.740 - 0.386(\log[\text{triallate}]) \qquad [4]$$

In the absence of triallate, HA had no significant effect on oat plant height (Fig. 30). The addition of HA (2.7 m*M* C) to nutrient solutions containing 0.1, 1.0 and 6.0 μM triallate resulted in significant increases in oat plant height (ranging from 36 to 58 % as compared with the no HA triallate treatments), and reductions in toxicity to oats

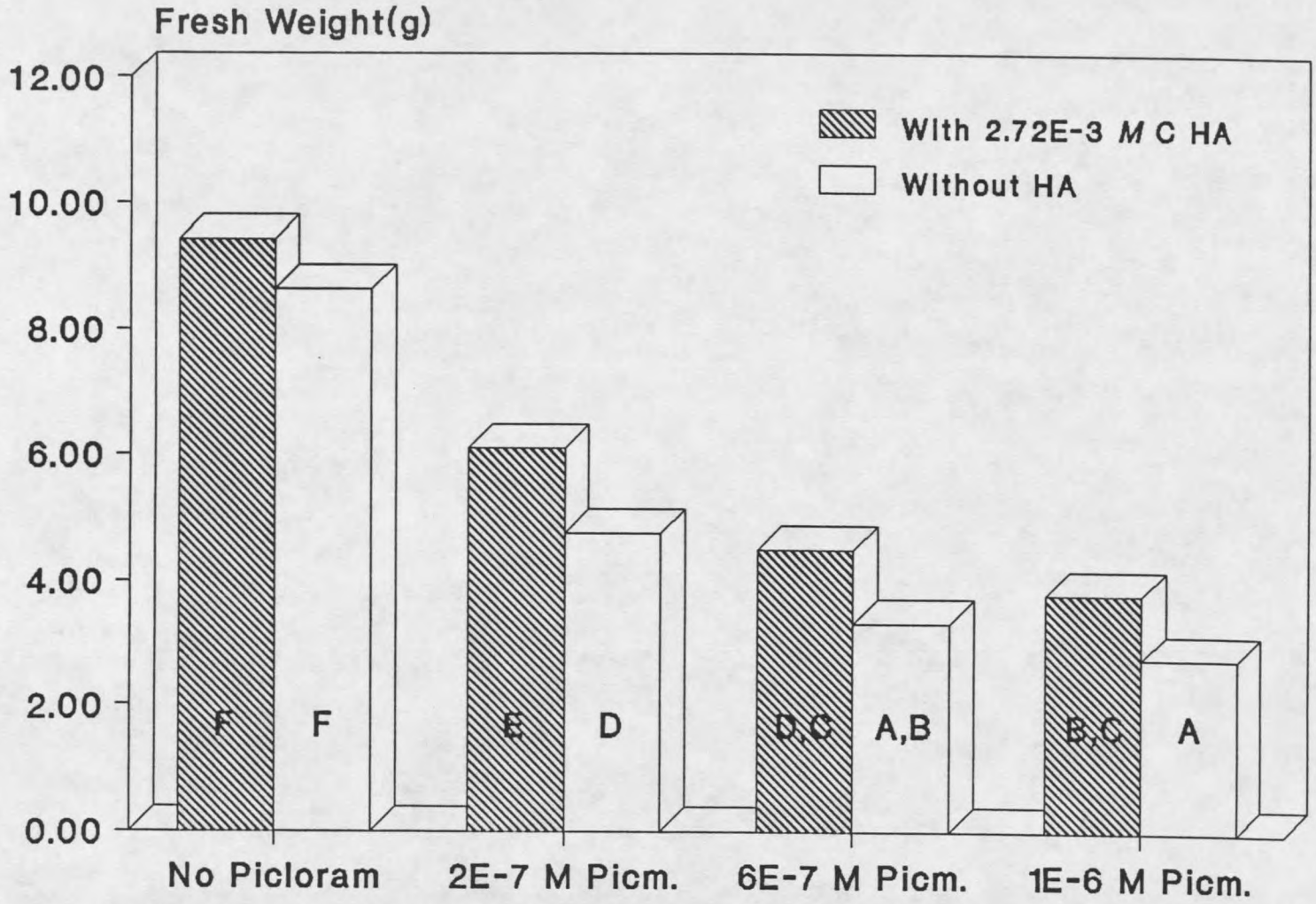


Fig. 28. Effects of Aldrich HA on the phytotoxicity of picloram to tomatoes. LSD = 0.828. Different letters indicate 90% significantly different groups. [HA] = 2.72 m*M* C.

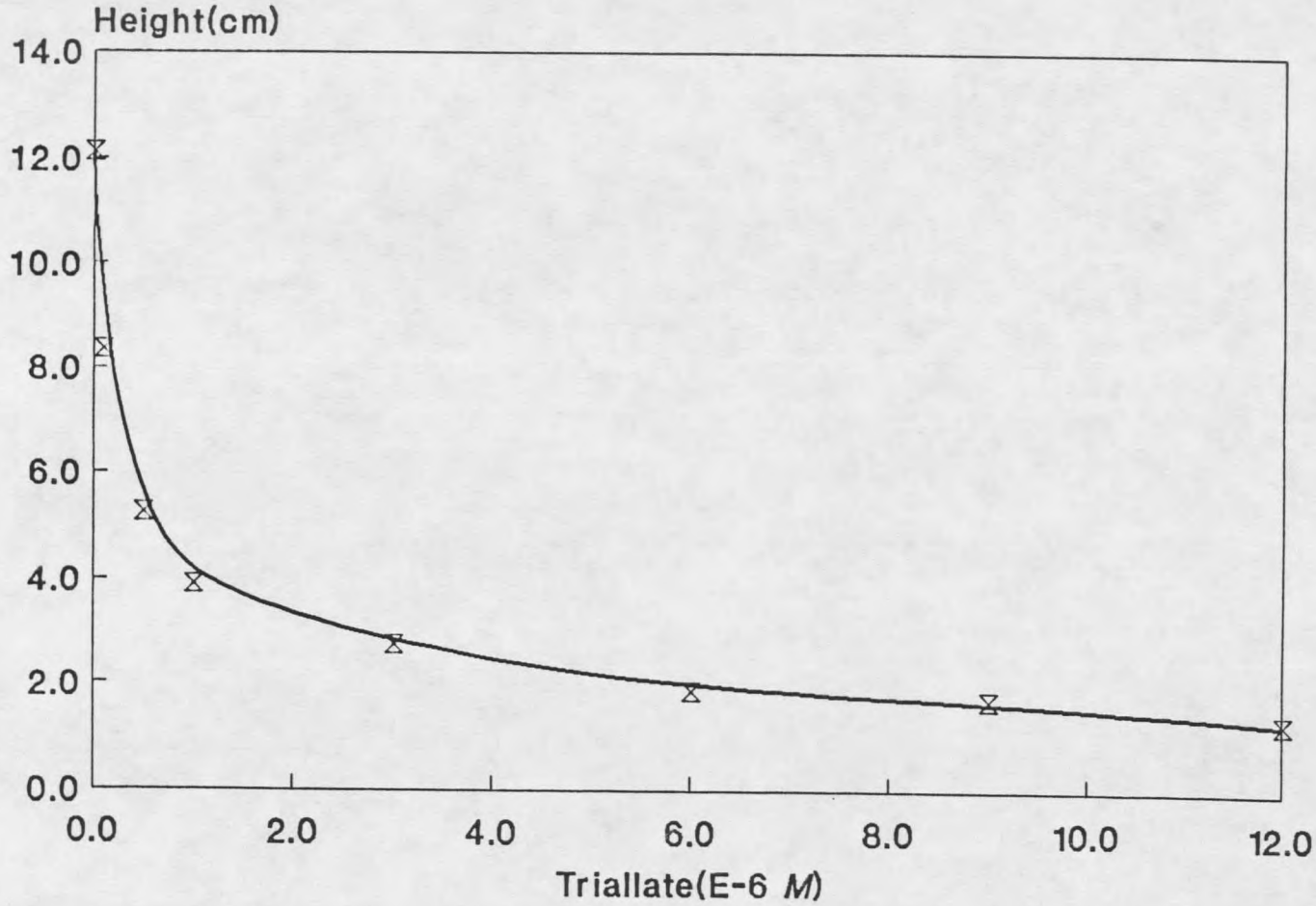


Fig. 29. Standard curve for triallate bioassay: whole plant fresh weight of oat plants vs. triallate concentration. The standard deviation (SD) for the data ranged from 0.412 to 2.512.

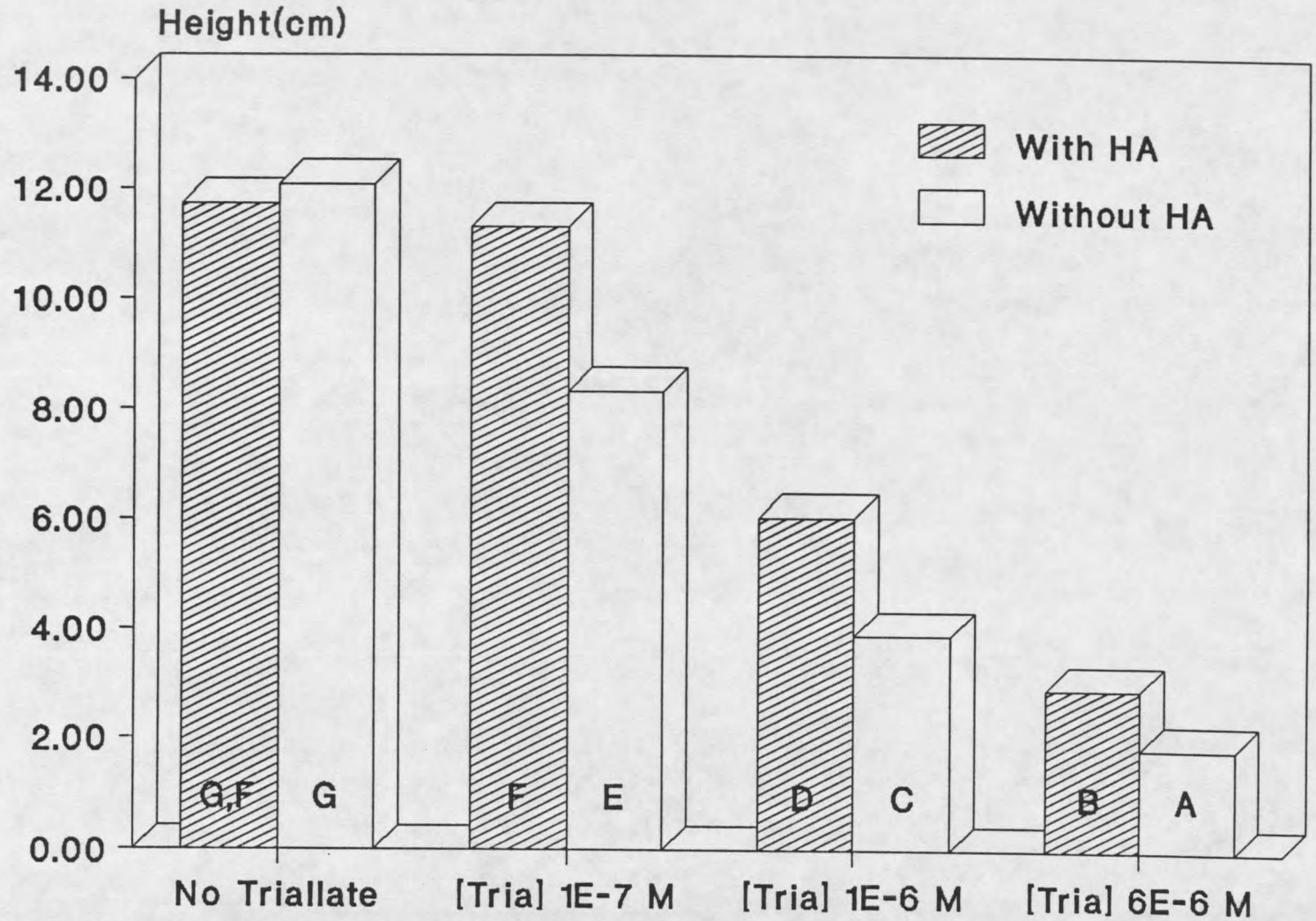


Fig. 30. Effects of Aldrich HA on the phytotoxicity of triallate to oats. LSD = 0.638. Different letters indicate 90% significantly different groups. [HA] = 2.7 m*M* C.

(Fig. 30). Calculations based on Eq. [4] revealed that the bioavailable concentrations of triallate were 0.06, 0.3 and 2.0 μM when the initial triallate concentrations in the HA nutrient solutions were 0.1, 1.0 and 6.0 μM, respectively.

HA-Herbicide Interaction and Bioavailability

The presence of HA resulted in an 18 to 39 % increase in the fresh weight of oats exposed to atrazine, a 28 to 38 % increase in the fresh weight of tomatoes exposed to picloram and a 36 to 58 % increase in the height of oats exposed to triallate over concentrations of atrazine, picloram and triallate ranging from 1.0 to 4.0, 0.2 to 1.0 and 0.1 to 6.0 μM, respectively (Fig. 25, Fig. 28 and Fig. 30). The ability of HA to reduce bioavailable concentrations of these herbicides followed the order: triallate > atrazine $\approx$ picloram. The water solubilities of picloram, atrazine and triallate are 430 (25 °C), 33 (27 °C) and 4 (25 °C) ppm, respectively. Since the interaction between nonionic organic solutes and humic substances is mainly hydrophobic complexation (binding) of the organic solutes with the hydrophobic regions of HA (Chiou et al., 1983, 1986), the strength of complexation is generally proportional to the hydrophobicity of the solute (Chapters 2, 3 and 4). Consequently, the predicted reduction in bioavailability of these herbicides in the presence of HA would increase in the order: triallate > atrazine > picloram. Our results are generally consistent with the predicted order, where triallate demonstrated the greatest reduction in bioavailability in the presence of HA. Observed results for atrazine and picloram were not entirely consistent with the expected trend based on the water solubility of the herbicides. However, the HA:herbicide concentration ratio was not constant for experiments with atrazine and picloram. Changes in herbicide

concentration at a fixed level of HA may result in different amounts of complexed herbicide and this would potentially influence the predicted order of reduction in herbicide bioavailability. In addition, the mode of action is different for atrazine and picloram; atrazine is a photosynthesis-inhibitor while picloram is a growth-regulator type herbicide. It is not clear that these two herbicides would necessarily have the same phytotoxicity even if the amount of atrazine and triallate complexed by HA were constant. Nevertheless, our results do indicate that reductions in bioavailability or herbicide efficacy in the presence of humic substances will increase with the hydrophobicity of the solute.

It is necessary to point out that our data reflected the periods when complexation of herbicide with HA and the subsequent reduction in bioavailability of the herbicide occurred. Complexation of herbicides by dissolved HA is a dynamic equilibrium, and the equilibrium will be disturbed if the concentration of the unbound herbicide decreases due to plant uptake. Bertin et al. (1990) found that 0.7 % of the radioactivity originally introduced was in maize plants grown in soil mixed with "natural" HA containing ^{14}C atrazine residue resulting from one year incubation under natural conditions, indicating that some of the bound atrazine residues slowly became available to plants.

Conclusions

Fresh weights of oats exposed to atrazine levels ranging from 1.0 to 4.0 μM were 18 to 39 % higher in the presence of atrazine plus HA (about 3 mM C) compared to

atrazine alone. The humic acid control showed little or no effects on fresh tissue weights. The length of herbicidal exposure time (2 - 6 days) did not influence the effects of HA on atrazine phytotoxicity to oats. When oats of different growth stages were exposed to an atrazine concentration of 0.5 μM for 2 days, the reduction in bioavailability of the herbicide was evident only when oat plants were younger than 21 days. The fresh weights of tomato plants receiving 0.2 to 1.0 μM picloram plus HA (2.7 mM C) were 28 to 38 % higher than those receiving picloram only, indicating a decrease in phytotoxicity in the presence of HA. Similarly, a reduction in oat phytotoxicity to triallate concentrations ranging from 0.1 - 6.0 μM was demonstrated by a 36 to 58 % increase in the height of oats in the presence of HA (2.7 mM C).

It is possible that HA may reduce phytotoxicity of herbicides by facilitating the metabolism of herbicide by the plant; however, at this time no literature is available to support this hypothesis. Further experiments with ^{14}C labeled herbicides would be required to elucidate the mechanism of phytotoxicity reduction caused by humic substances. However, given the fact that other studies have measured significant complexation of organic solutes with dissolved humic substances, we hypothesize that the complexation of atrazine, triallate and picloram with dissolved HA reduced the herbicidal injury to oats and tomatoes. This suggests that complexation of herbicides with soluble C may play an important role in the bioavailability and toxicity of organic compounds in natural waters.

CHAPTER 6

SUMMARY

Dissolved humic substances are ubiquitous in soils, sediments and natural waters. Other studies have demonstrated that interactions between humic substances, especially humic and fulvic acids, and hydrophobic organic solutes result in (i) enhancement of the apparent water solubility of nonionic solutes, (ii) reduction in volatilization of volatile organic solutes, (iii) a decrease in the adsorption of organic solutes to solid phases and (iv) a decrease in the bioavailability and toxicity solutes. Consequently, the behavior and fate of organic solutes in the environment may be significantly influenced by the presence of humic substances.

The complexation of several pesticides with dissolved humic and fulvic acids was studied using fluorescence quenching, fluorescence lifetime measurements, static headspace gas chromatography and bioassay techniques. Fluorescence quenching of a fluorescent solute molecule in the presence of humic acid may result from either the formation of solute-humic complexes (static quenching) or the collisions of solute and humic molecules as a result of diffusion processes (dynamic quenching). Fluorescence quenching is generally limited to studying solutes that fluoresce, however, humic and fulvic acids are also fluorophores with low quantum yield, and quenching by nonfluorescent solutes may be useful for studying complexation. Static headspace analysis relies on the Henry's law relationship between the activity of solutes in the aqueous phase and the partial pressure of the solute in the gaseous phase. Complexation of volatile

solutes by humic substances reduces the activity of the solute in the aqueous phase, and the resultant gaseous phase concentration decreases as well. The major limitation of headspace GC analysis is that the solute must be volatile with a vapor pressure exceeding 10^{-4} mm Hg at room temperature.

Relatively little is known about the phytotoxicity of organic solutes (e.g. herbicides) in the presence of humic or fulvic acids. If the complexation of organic solutes with humic substances reduces their toxicity, then bioassay techniques may be useful for assessing the amount of complexation of organic solutes. Furthermore, given that complexation of nonionic solutes by HA is an important process in natural systems, it is important to determine whether these complexes are bioavailable.

Fluorescence quenching studies with fluoranthene, 1-naphthol and napropamide showed significant complexation of these solutes with a variety of dissolved humic and fulvic acids. Results from temperature dependence, lifetime measurements and viscosity experiments all suggested that the primary quenching mechanism was static, resulting from the formation of solute-humic complexes. Conditional complexation constants ranged from 9.7 to 91.5 L/g C for these solutes and generally increased with increasing solute hydrophobicity.

Similar results were obtained with static headspace GC analysis for herbicides dichlobenil, triallate and trifluralin. Conditional complexation constants ranged from 5.9 to 45.7 L/g C and generally increased with increased solute hydrophobicity. The results from fluorescence quenching and headspace analysis indicate that significant fractions of

hydrophobic nonionic solutes will form soluble complexes with humic substances in natural systems.

Bioassay studies were conducted using indicator plant species with the herbicides atrazine, picloram and triallate to ascertain the bioavailability of herbicides complexed with humic acid. The presence of humic acid at level ranging from 2 - 3 m*M* C reduced the phytotoxicity of these three herbicides to indicator species. These results demonstrate that complexation of herbicides with soluble humic substances may influence bioavailability in natural systems.

The complexation of nonionic solutes by humic substances observed in this study is consistent with other studies showing a hydrophobic interaction or partitioning mechanism. The results also demonstrate the importance of solute-humic interactions for understanding the fate and behavior of organic compounds in soils. Given the number of hydrophobic solutes which exist as potential pollutants in natural systems, it is clear that further systematic studies are necessary to increase our appreciation for the role of soluble humic-solute complexes in environmental processes.

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