This is to certify that the

thesis entitled

LITTORAL-PELAGIAL INTERACTIONS:

THE ROLE OF DISSOLVED HUMIC MATERIALS

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D degree in Botany

Major professor

Date 13 November 80

LITTORAL-PELAGIAL INTERACTIONS:

THE ROLE OF DISSOLVED HUMIC MATERIALS

by

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A DISSERTATION

Submitted to Michigan State University
In partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1980

ABSTRACT

LITTORAL-PELAGIAL INTERACTIONS:
THE ROLE OF DISSOLVED HUMIC MATERIALS

By

Arthur Jay Stewart

Relationships between ultraviolet absorbance, fluorescence, and concentrations of dissolved organic carbon (DOC) were examined in four model humic materials. Fluorescence was consistently a poor predictor of DOC concentration, and absorbance correctly predicted DOC concentration only in the more labile materials. Asymmetry between DOC and the two optical parameters was related to dissolved organic matter (DOM) apparent molecular weight, and could be explained by greater levels of internal quenching and shielding in compounds of larger apparent molecular weight. A lake-to-lake comparison demonstrated a calcium-related selective loss of high molecular weight dissolved humic material (DHM), which invalidates the use of either optical parameter as a good predictor of DOC concentration in hardwater systems. However, fluorescence-absorbance ratios may be useful in delineating seasonal and depth distribution patterns of low and high molecular weight DHM within a given lake.

Under controlled conditions, sediment/pH state markedly influenced the apparent molecular weight spectrum of selected DHM. Field and laboratory data indicate that organic-rich sediment absorbs DHM of high apparent molecular weight leaching from scenscing littoral vegetation during rain events, and slowly releases DHM of lower apparent molecular weight between rain events. Sunlight and high-intensity UV light were

sufficient to cause rapid (< 300 minutes) alteration in the spectral characteristics of DHM, and solar radiation is likely important in seasonal regulation of DHM quality in shallow waters and in the upper photic zone in pelagial areas. Low concentrations (< 2 mgl^{-1}) of fulvic material were found to be potent inhibitors of the $CaCO_3$ precipitation process under natural conditions, but did not significantly alter short-term (0-3 days) rates of ^{14}C assimilation by natural algal assemblages. Binding of orthophosphate to DHM could not be demonstrated under conditions similar to those in the epilimnion of the study site, but is potentially an important regulator of phosphate in systems of lower alkalinity.

Mixed natural assemblages of algae and bacteria generally demonstrated lower rates of ^{14}C assimilation and high rates of dissimilation of recent photosynthate when amended with low concentrations (7.2 mg l^{-1}) of unfractionated DHM, and the extent of the inhibition or stimulation was greatest in the smaller (1-5 μm) assemblage particles. In different assemblage types, additions of DHM markedly enhanced community alkaline phosphatase activity (APA), particularly under low-light regimes. DHM of low apparent molecular weight was much more stimulatory to both ^{14}C assimilation and APA than was DHM of high apparent molecular weight, and higher concentrations of DHM (either unfractionated, or molecular weight fractionated) produced greater APA responses than did lower concentrations. Addition of phosphate enhanced the disparity in rates of ^{14}C assimilation of

samples incubated under low and high light regimes, increased rates of ^{14}C assimilation, and depressed APA. Interactions between DHM and phosphorus were indicated in several experiments. Two hypotheses were invoked to explain increases in APA in response to DHM: (1) increased competition between algae and bacteria for phosphate following bacterial release from substrate limitation, or (2) DHM may have acted as a sequestering agent for organophosphorus compounds, and in so doing, gradually depleted phosphate availability. In either case, it is clear that DHM in some manner alters phosphorus cycling. This DHM characteristic may be ecologically as important as its ability to complex trace metals.

The proportion of APA directly affiliated with photosynthetic organisms can be determined by comparing distribution of APA and assimilated carbon in samples following gentle centrifugation. In four lakes in southern Michigan, algal APA contributed only 4-30 percent to total APA, which is much lower than expected based on numerous other studies.

"A man who uses an imaginary map, thinking it to be a true one,
is likely to be worse off than someone with no map at all;
for he will fail to inquire wherever he can, to observe
every detail on his way, and to search continuously
with all his senses and all his intelligence for
indications of where he should go."

- E. F. Schumacher

To my parents - who were careful to withhold the map.

ACKNOWLEDGEMENTS

The Kellogg Biological Station is a rough and peculiar patchwork of serenity and chaos; with a little specialization in practical island biogeography, it is an environment perfectly suited to the development of good science from outlandish ideas by young students. It was for me.

Thanks must go to my committee members, Drs. R. B. Wetzel, E. E. Werner, P. A. Werner and P. G. Murphy for their helpful suggestions and critical evaluation of this manuscript. In particular, however, I owe a special thanks to Dr. Wetzel, who tolerated my criticism, listened to my most absurd ideas with patience, and allowed me to plunge on into areas never outlined in my proposal. Limnology still holds a magic.

Interactions with numerous individuals during the past several years proved invaluable; chief among these were the many hours of argument with Bill Crumpton, as we persued the elusive six-and-a-half minute mile on our late afternoon 10,000 meter runs. Other useful discussions were enthusiatically contributed by Gary King, Jim Grace, Dave Francko, Joyce Dickerman, Wilson Cunningham, Sven Beer, Derek Lovley, Amy Ward and Steve Weiss. I wish to extend appreciation to Anita Johnson for her flawless graphics, and to Jay Sonnad and Pat Brown, for their conscientious technical assistance generously donated on endless occasions. I am, in addition, grateful for the advice and assistance freely given by other members of the Kellogg Biological Station, particularly by Art Weist, Char Seeley, Mary Shaw and Marilyn Jacobs, Dolores Haire, John Gorentz and Dr. George Lauff.

Finally, no one did more than Joyce Dickerman, who shared in my elation when things worked, and in my despair when they did not. I offer my warmest acknowledgements to her, and to my daughter Joelle, who made this past year such an interesting challenge. Both I love dearly.

Financial support for these studies was provided in part by the Department of Energy (EY-76-S-02-1599, COO-1599-188).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES--continued

LIST OF FIGURES

LIST OF FIGURES--continued

CHATPER I

INTRODUCTION

Many lakes contain copious amounts of dissolved humic material (DHM), occasionally in quantities sufficient to impart a deep brown stain to the water. Other lakes, often in close geographical proximity to heavily stained systems, can contain less than 1 mg per liter of DHM, and have water that is exceptionally clear and transparent. This disparity raises two types of questions: First, what factors are important in regulating the quantities and quality of DHM in lakes? Secondly, what are the consequences of inputs of DHM to populations of pelagial microflora?

Answers to both of these questions are likely dependent upon the chemical nature of DHM. However, the chemistry of DHM is largely unknown, primarily because of the diversity and complexity of the constituent molecules. DHM characteristics that are generally agreed upon include the following:

1. DHM is chemically complex, and ranges in molecular weight from a few hundred to hundreds of thousands of daltons. DHM is a polyheterocondensate of carboxylic and phenolic acids; p-hydroxybenzaldehyde, vanillin and syringaldehyde are the dominant products found following chemical degradation.

2. Labile portions of the soluble organic compounds of decomposing plants and animals are utilized rapidly by decomposer organisms, and DHM is what remains. Consequently, DHM is considered to be relatively

resistant to further microbial degradation. As a corollary to this, the majority of the dissolved organic carbon (DOC) in lakes is DHM, since the pool size of labile material is small due to rapid utilization.

3. Many dissolved humic materials are good chelation and complexation agents, and may be ecologically important through regulation of rquired trace metals, such as iron and manganese.

4. Finally, DHM has several important spectral characteristics; it is brown or yellowish in color, and absorbs light strongly, particularly in the ultraviolet portion of the spectrum. In addition, DHM readily fluoresces by absorbing light of relatively short wavelengths and emitting photons of longer wavelength and lower energy.

In Lawrence Lake, and in may other small or moderate-sized lakes, production of DHM occurs predominately in the littoral zone or drainage basin, for synthesis of recalcitrant organic materials (lignin, hemicellulose, cellulose) originates most extensively in emergent macrophytes and terrestrial vegetation growing in the drainage basin. During and following intense rains, DHM produced from partial degradation of plant remains is leached from scenescing plant tissues and is moved rapidly from the site of its production out into the lake.

In this context, DHM may be considered to have ectocrine characteristics. DHM is produced in one area of the lake or drainage basin, is modified to some extent as it is moved to the lake from its site of production, and exerts, or potentially exerts, an influence on metabolic processes in other areas of the lake. A more specific question then becomes: What changes occur in DHM as it moves from the site of production to the epilimnion of the lake, and what are the consequences of DHM inputs to pelagial metabolism?

In this dissertation, I attempt to answer this question using a combination of field and laboratory studies. The investigations consist of three functional units: (1) A development of methods used to quantify the nature of DHM (Chapters II and III), (2) an elucidation of some abiotic factors important in regulating DHM concentration and quality (Chapter IV), and (3) a study of some of the consequences of inputs of DHM on natural assemblages of pelagial microflora (Chapter V). Finally, I have included an additional brief segment (Chapter VI) in order to explore some of the difficulties incurred in determining the ecological importance of one of the two assemblage response parameters that I selected for use in Chapter V.

CHAPTER II

Fluorescence-absorbance ratios: A molecular weight tracer of dissolved organic matter

INTRODUCTION

The ultraviolet spectral characteristics of naturally occurring aquatic humic substances have been studied by a number of investigators (Armstrong and Boalch 1961; Ogura and Hanya 1966, 1967), and have led to predictions of concentrations of dissolved organic matter based on optical density values at one or more wavelengths (Dobbs et al. 1972; Banoub 1973; Lewis and Tyburczy; Lewis and Canfield 1977). More recently, fluorescence of dissolved humic substances has been employed as a measure of dissolved organic carbon (Christman and Minear 1971; Smart et al. 1976) and has been used as a tracer of water movements in lakes (Spain and Andrews 1970).

When humic substances are molecular weight-fractionated by one means or another, differences in the absorbance or fluorescence characteristics of the various molecular weight fractions can be found (Ghassemi and Christman 1968; Levesque 1972; Tan and Giddens 1972). In particular, low molecular weight materials fluoresce more intensely than do high molecular weight humic substances. While the reasons for this fluorescent behavior are not clear, this characteristic may be used to monitor shifts in the molecular weight spectrum of dissolved humic materials.

This study explores the ability of coupled absorbance-fluorescence parameters to depict vertical and seasonal distribution patterns in

molecular weight classes of dissolved humic substances. A convenient means of estimating relative molecular weight of dissolved organic carbon would enhance understanding of processes that regulate the distribution and abundance of these particular forms of dissolved organic carbon in aquatic ecosystems.

MATERIALS AND METHODS

Absorbance values were obtained at 250 nm in 1.0-cm matched quartz cuvettes with a Hitachi Perkin-Elmer UV-VIS spectrophotometer model 39. All fluorescence measurements were made using a Turner model 110 fluorometer with a primary filter peaking at 360 nm and secondary filters (Turner specification numbers 2A and 48) selected to pass wavelengths of 460 nm. The fluorometer was standardized against a 15 $\mu g\ l^{-1}$ quinine sulphate solution, which gave 30 fluorescence units at a setting of 10×. Fluorescence readings were either taken at a setting of 3× or were corrected to corresponding 3× values after reading samples at 1×. Absorbance and fluorescence intensities of all samples were determined at room temperature after samples had been filtered through precombusted 0.5-μm pore size Reeve Angel 984-H glass fiber filters under a vacuum differential of 0.5 atm. Sample pH was determined with a combination electrode and a Coleman model 38A pH meter.

In some experiments molecular weight fractionation of the humic materials was accomplished using gel permeation chromatography (Sephadex G100/120) prepared according to manufacturer's instructions. A bed volume of 22.7 cm^3 (1 × 29 cm) was used so that small sample quantities could be processed rapidly. Tubes in which the fractions

were to be collected were weighed to the nearest 0.01 g before and after fraction collection for correction of small differences in elutant volumes (V_e). Elutant buffers (either 0.025 M borax-HCl at pH 8.10 or 0.025 M borax-NaOH at pH 9.45) were selected to minimize solute-gel interactions (Swift and Posner 1971). The void volume of the column (V_0) was determined with blue dextran 2000, while total column volume (V_t) was calculated geometrically. A flow rate of 13.8 ml $cm^{-2}h^{-1}$ was used throughout the experiments. After collection, each fraction (ca. 0.75 ml) was diluted with 5.0 ml of distilled-deionized water before fluorescence or absorbance values were determined. Corrections for buffer absorbances were made wherever appropriate. In several experiments humic materials collected as described below were fractionated using dialysis tubing with a nominal molecular weight cutoff of 3,500 daltons.

Dissolved humic material was concentrated from Lawrence Lake, Barry County, Michigan on Amberlite XAD-2 macroreticular resin at pH 2.2 using the technique described by Mantoura and Riley (1975). Other samples of humic material were collected in a similar way from more deeply stained, wooded ponds, or from leachate from emergent wetland plants Typha or Carex. Materials bound on the resin were eluted with a 50/50 (v/v) mixture of 2.0 M NH_4OH and methanol, brought to dryness at 35°C under reduced pressure, reconstituted in distilled-deionized water, and lyophilized prior to further analyses. No differences were found in optical characteristics of unconcentrated vs. lyophilized, reconstituted samples. The optical properties of the samples were stable for several days at room temperature.

RESULTS AND DISCUSSION

When dissolved humic material of water from Lawrence Lake was fractionated at pH 9.45 on G100/120, absorbance and fluorescence peaks differed. In every case, the peak in fluorescence emerged behind the peak in absorbance by 1.50 to 2.25 ml. An identical pattern occurred when the borax-HCl buffer system (pH 8.10) was used (Figure 1). Almost identical results were found for concentrated Typha leachate and for fulvic and humic acids isolated from Lawrence Lake sediments. A plot of [fluorescence (1×):absorbance at 250 nm] vs. V_e/V_t demonstrated the presence of two zones (Figure 2). In Zone I, the higher molecular weight fractions had fluorescence (1×):absorbance$_{250}$ ratios (f:A) which approached 200-250. After about 21 ml were eluted, Zone II emerged as fractions with rapidly increasing f:A values. The smaller molecular weight fractions showed a spectrum of f:A values from about 250-600. While estimation of precise molecular weights of the two fractions is subject to considerable error (Cameron et al. 1972), the data substantiate that the fluorescent nature of the materials per unit absorbance was greater for smaller molecular weight compounds.

When samples collected with the XAD-2 resin were exhaustively dialyzed against distilled deionized water, two fractions were obtained. Fractions with an apparent molecular weight of less than 3,500 were of a pale straw color, had relatively low absorbance values, and fluoresced intensely. Fractions with apparent molecular weights greater than 3,500 were darker in color (often with an olivaceous cast), absorbed strongly at 250 nm, but exhibited low fluorescence capacity (Figure 3). Similar fractionation patterns were found for fresh leachate material from Typha, Carex, and leaf litter, and demonstrated that the XAD-2 resin extraction procedure did not result

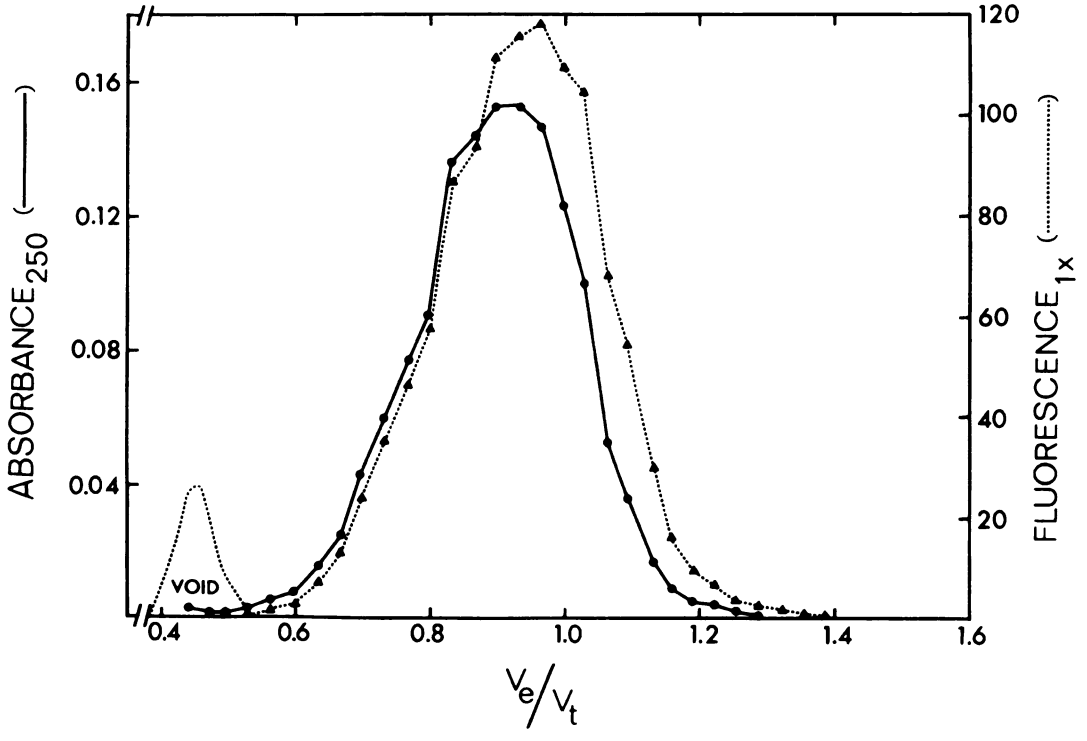


Figure 1. Sephadex G100-120 fractionation of humic materials collected with XAD-2 resin from Lawrence Lake inlet water. Borate-HCl buffer, pH 8.10; flow rate, 13.8 ml cm^{-2} h^{-1}. Volume eluted per fraction is 0.74 $\pm$ 0.05 ml.

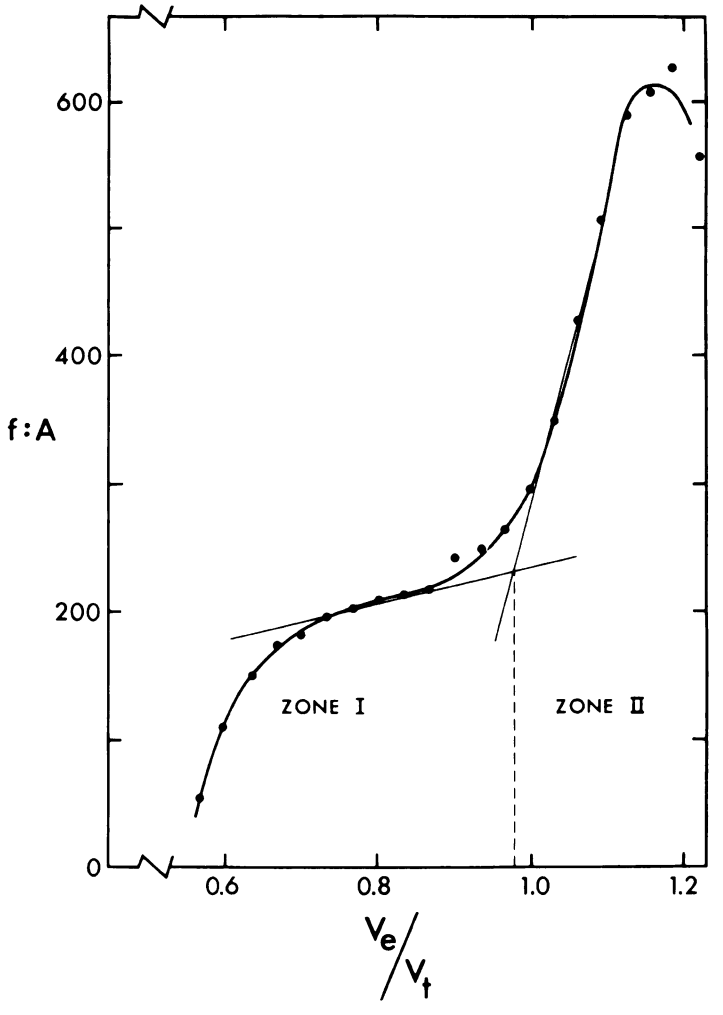


Figure 2. Segregation of low f:A (zone I) and high f:A (zone II) humic materials on Sephadex G100-120.

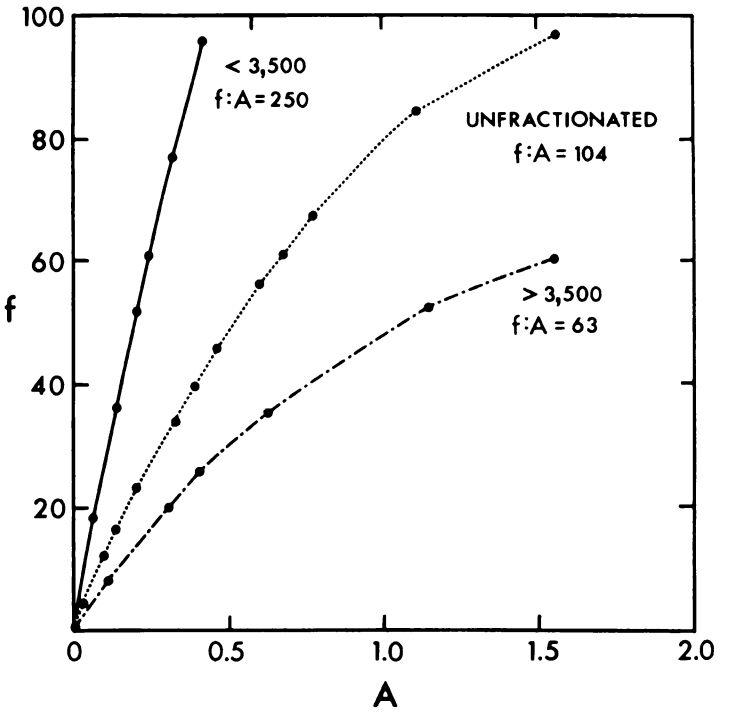


Figure 3. Fluorescence (1X):absorbance (250 nm, 1-cm cells) relationships for unfractionated (·····) and dialysis-fractionated (apparent molecular wt < 3,500, ——— ; > 3,500, -·—·—·—·) humic materials. Values of f:A calculated from slopes below absorbance = 0.40.

in the dialysis experiment f:A shifts (Table 1).

Intensity of fluorescence is pH dependent (Ghassemi and Christman 1968). The pH-fluorescence response curves for young and aged Typha leachate and from humic material collected from Lawrence Lake on XAD-2 resin were similar (Figure 4), with maximal fluorescence intensities occurring at pH 9 or 10. Over a pH range from 4.5 to 10.1, $absorbance_{250}$ increased by less than 3.5%. pH-induced absorbance and fluorescence changes introduced relatively small errors into the calculated f:A parameter, but if sample-to-sample pH values differ significantly, appropriate corrections are necessary.

A synoptic survey of 24 littoral and 13 pelagial sites of Lawrence Lake on a still spring morning yielded f:A ratios of 331.6 $\pm$ 1.3 (SE) and 328.5 $\pm$ 1.6 (SE), respectively. Water entering the lake through the main inlet, which supplies a majority of the water inputs to the lake during the ice-free period and drains a large wetland marsh (Wetzel and Otsuki 1974), had a f:A ratio of 509.7 $\pm$ 8.7 (n=15). These data suggest that one or more mechanisms are operating in the lake to decrease the f:A ratio.

Depth-time isopleths of f:A ratios in Lawrence Lake (Figure 5) show that f:A ratios were high in deeper waters under ice cover, minimal in the epilimnion during summer stratification, and maximal in the hypolimnion in late summer. The spatial displacement of the summer maximal and minimal values corresponds to the period of epilimnetic decalcification and suggests that low molecular weight fractions may be selectively removed from the epilimnion through co-precipitation with calcium carbonate and accumulate in hypolimnetic waters as $CaCO_3$ is re-solublized. Alternatively, the observed f:A distribution may result

Table 1. Representative f:A values for various parent humic materials and their dialysis-fractionated components. Dialysis nominal molecular weight cutoff = 3,500.

Source of parent material	Treatment	Parent material	<3,500	>3,500
Lawrence Lake, subsurface pelagial	XAD-2 concd	210	230	149
Lawrence Lake, main inlet	XAD-2 concd	207	330	159
Darkly stained roadside ditch	XAD-2 concd	104	250	63
Carex leachate	XAD-2 concd	99	170	84
Carex leachate	lyophilized	82	156	56
Typha leachate	lyophilized	84	139	63
Leaf litter leachate	unconcd	59	165	58
Purdy Bog	unconcd	84	131	65
Cassidy Lake	unconcd	133	N.A.	102

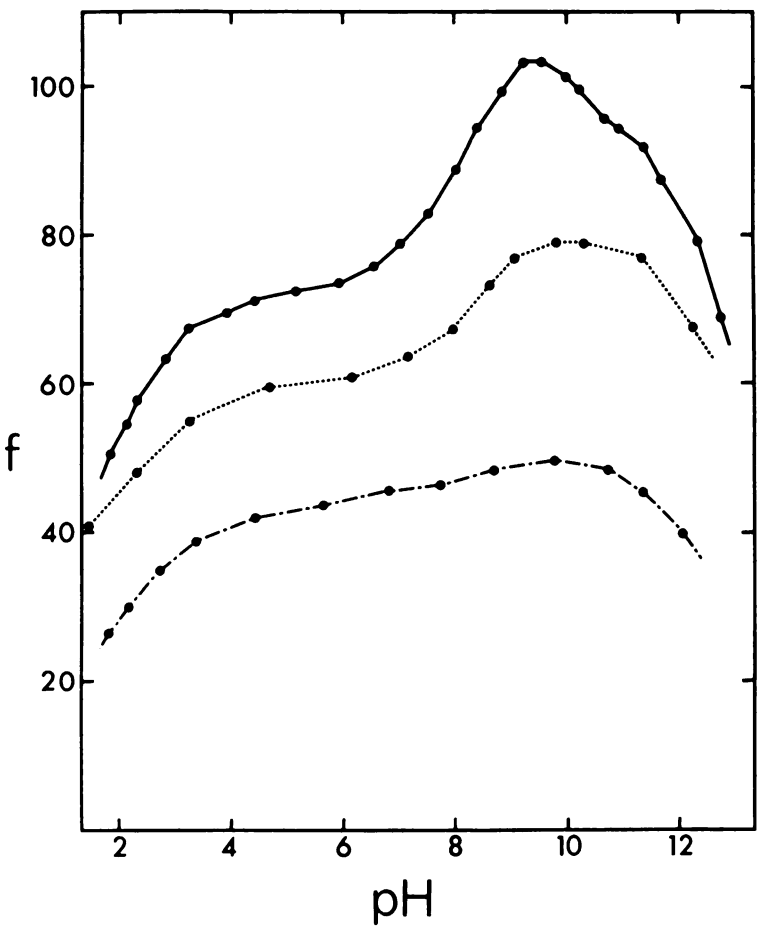
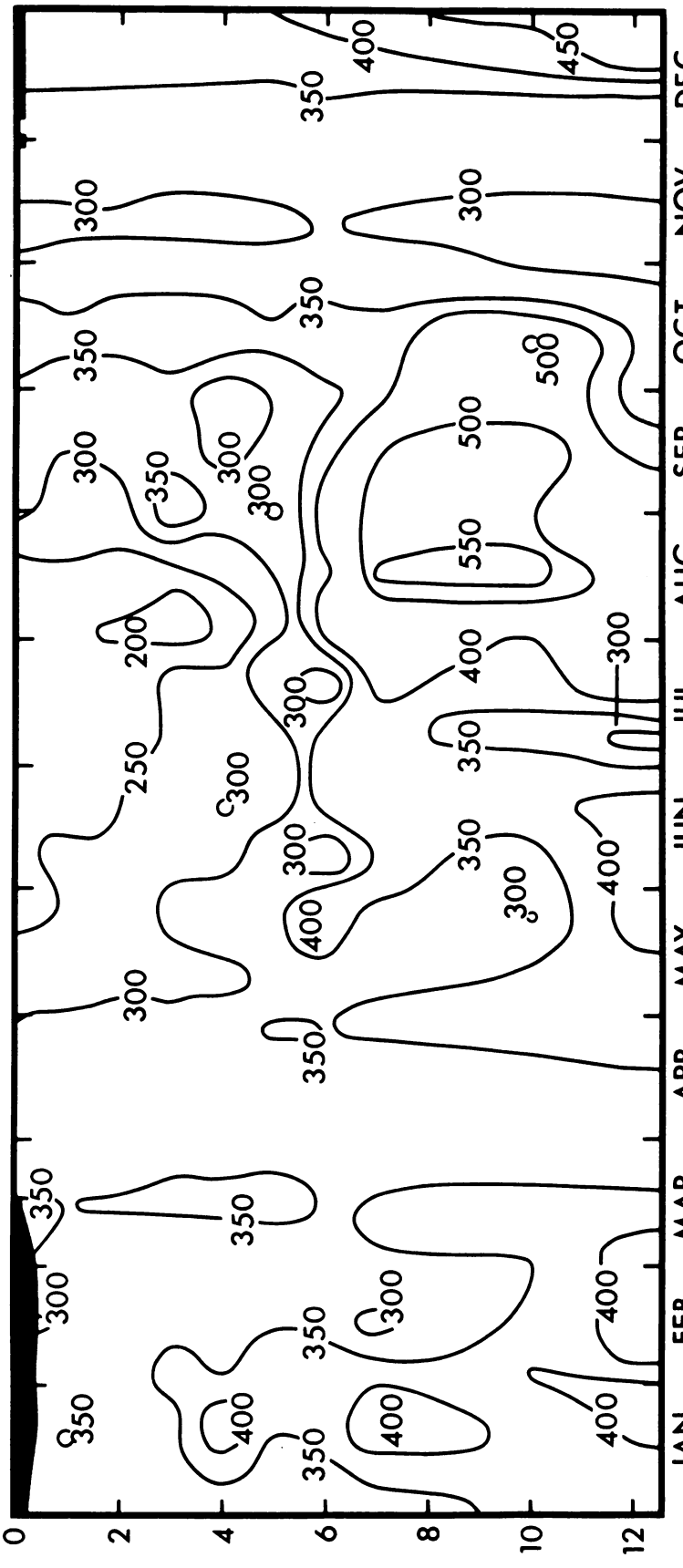


Figure 4. pH-fluorescence response curves for young Typha leachate (——————), aged Typha leachate (·····), and humic materials (—·—·—) collected from Lawrence Lake inlet water with XAD-2 resin.

Figure 5. Depth-time diagram of isopleths of f:A ratios in Lawrence Lake, Michigan, 1977. Opaque areas--ice cover to scale.


DEPTH (m.)
0
2
4
6
8
10
12
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
1977

from differences in rates of in situ production or utilization of fluorescent or absorbant dissolved organic matter components. The role of calcium in the distribution of f:A patterns has been implicated in several other stuides (Otsuki and Wetzel 1973, 1974; Wetzel and Otsuki 1974, and is currently under investigation.

The capacity of the f:A ratio to delineate relative distribution patterns of low and high molecular weight dissolved humic materials depends upon the linearity of fluorescence and absorbance values to increasing concentrations of the dissolved humic compounds. Ideally, the proportion of fluorescing or absorbing substances of humic and non-humic origins, and substances which interfere with the fluorescing capacity of humic materials, should be evaluated.

The linear relationship between fluorescence and absorbance with changes in concentration degrades if very strongly absorbing samples are analyzed (Figure 3). Non-linearity of fluorescence is more pronounced in samples with low f:A values, and can be corrected by making serial dilutions of the sample. Plots of f:A vs. A or, alternatively, of f vs. A are desirable, and f:A values are best calculated only over the linear portion of the plot. Experiments with a number of samples and dilutions suggest that the f:A ratio is representative of relative molecular weight if, for any sample or dilution, fluorescence at a setting of 3 × is greater than 10 while absorbance (250 nm, 1-cm cells) is less than 0.50 (Figure 3).

Fluorescence and absorbance measurements alone probably cannot fully quantify the DOC pool of a lake, since many dissolved organic compounds neither absorb at 250 nm nor fluoresce. Consequently, the relationship between total DOC and absorbance is likely to vary from

lake to lake and perhaps from season to season. Evidence from this study indicates that fluorescing and UV-absorbing dissolved organic fractions are of humic origin, and f:A measurements may be extremely useful in depicting changes in at least the humic portion of the DOC pool. Some bacteria, however, produce fluorescing compounds (Wasserman 1965; Caldwell 1977) which, in hypereutrophic or meromictic lakes, could lead to difficulties in interpretation of f:A ratios. In a majority of lakes of low to intermediate trophy, bacterial production of non-humic fluorescence should be minor in comparison to humic substance fluorescence.

Iron, copper and other transition metals at high concentrations can alter the fluorescence capacity of humic materials (Ghassemi and Christman 1968; Levesque 1972). Some inorganic materials (e.g. nitrite, borate) absorb in the ultraviolet portions of the spectrum, but interferences are largely avoided at 250 nm (Armstrong and Boalch 1961; unpublished data). Bias introduced into the f:A parameter by these factors should be evaluated in interpreting lake-to-lake f:A differences. The application of f:A ratios as a general index of seasonal or vertical distribution of low and high molecular weight dissolved humic species within any given lake can be extremely useful.

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CHAPTER III

Asymmetrical relationships between absorbance, fluorescence, and dissolved organic carbon

INTRODUCTION

There have been numerous attempts to use absorbance or fluorescence characteristics of dissolved organic matter (DOM) to estimate concentrations of dissolved organic carbon (DOC), primarily because these two optical parameters can be measured quickly and accurately, and because measurements of this nature are nondestructive (e.g. Banoub 1973; Lewis and Tyburczy 1974; Smart et al. 1976; Lewis and Canfield 1977). The potential advantages of being able to determine concentrations of DOC using either fluorescence or absorbance measurements are considerable, and led me to examine more closely relationships between DOC, fluorescence and absorbance. In this study I used a combination of laboratory and field analyses to delineate conditions under which either of the two optical characteristics can be most appropriately used as measures of DOC.

MATERIALS AND METHODS

The DOM used in the experimental portions of this study consisted of leachate obtained from leaves of uniformly ^{14}C-labelled water lily (Nuphar variegatum), leachate obtained from the leaves of uniformly ^{14}C-labelled cattail (Typha latifolia), humic materials resulting from partial decomposition of ^{14}C-labelled T. latifolia leachate, and Kraft

indulin AT lignin.

Nuphar variegatum was grown in an atmosphere of $^{14}CO_2$ for 2.1 weeks (gas renewed every 2 days), and labelled leaves were harvested and lyophilized. Portions of the dried leaves were finely ground and mixed with 100 ml of Millipore Ultrapure "Q" water and inoculated with 0.5 ml of naturally occurring littoral microflora obtained from Lawrence Lake, Barry County, Michigan. The resulting slurry was left in an open beaker, and was occasionally swirled to resuspend and aerate the contents. After one week, 8 ml of the supernatant was filtered through a 0.5-µm pore size (Sheldon 1972) Reeve Angel (984-H) glass fiber filter, and the filtrate was immediately frozen, lyophilized and stored under desiccant until use.

^{14}C-labelled T. latifolia was obtained by pulse-labelling T. latifolia plants with $^{14}CO_2$ in the field 10 times during a growing season (Dickerman and Wetzel, in prep.). The T. latifolia plants were allowed to senesce naturally, and the dried senescent shoots were harvested, fully desiccated under silica gel, and ground to a coarse powder with a Wiley mill. Leachate was then obtained from the ground tissues in the same way as that of N. variegatum leachate.

Humic materials were derived by decomposing portions of the labelled T. latifolia leaf powder in 250 ml of Millipore Ultrapure "Q" water (1.0 ml lakewater inoculum) in a glass-stoppered flask for 12 months. The slurry was intermittantly aerated with oxygen to insure that some aerobic decomposition could occur. At the end of the 12-month period, pH of the material was adjusted to 12 with NaOH and the contents of the flask were shaken on a gyratory shaker for 24 h. The intensely colored supernatant was filtered through a 0.5-µm pore size glass fiber

filter, and the filtrate was adjusted to pH 2.2 with HCl. Humic materials were trapped on a column of Amberlite XAD-2 macroreticular resin and eluted as their ammonium salts using a 50/50 (v/v) mixture of 2 M NH_4OH and methanol (Mantoura and Riley 1975a). The eluant was brought to dryness at 35°C under reduced pressure, reconstituted in 10 ml of "Q" water, and lyophilized. The ammonium humate was stored under desiccant until use.

Kraft indulin AT lignin was obtained from Westvaco, Inc., and was used without further preparation.

Gel permeation chromatography (GPC) of the four organic materials was accomplished using Sephadex G100-120 prepared as per manufacturer's instructions. The void volume (V_o, 8.5 cm^3) of the column was determined using Blue Dextran 2000, and total column volume (V_t) was calculated geometrically (1 × 29 cm). Materials were eluted with 0.025 M borate buffer adjusted to pH 8.60 with HCl at a flow rate of 0.74 ml cm^{-2} min^{-1}. Fractions (0.58 $\pm$ 0.04 ml SD; n = 100) were collected in tubes that were weighed to the nearest 0.01 g before and after fraction collection to correct for small differences in eluant volume (V_e). Portions of each of the four organic materials were reconstituted in 3-4 drops of the borate buffer immediately prior to use to preclude gel-DOM interactions resulting from solvent-eluant differences (Söchtig 1972).

Eluted labeled carbon was determined for each fraction by adding 200 or 250 µl of the fraction to 10 ml of scintillation media (Instagel), and radio-assaying with a liquid scintillation counter (Beckman LS8000). Background and quench corrections were made before correcting to DPM. DOC concentrations of eluted Kraft lignin fractions were determined using a slight modification of the technique outlined by

Menzel and Vaccaro (1964). 200 or 250 µl subsamples of each eluted fraction were diluted with 4.0 or 4.5 ml of "Q" water prior to spectrophotometric assays of fluorescence and absorbance. Absorbance at 250 nm (A_{250}) was determined with a Hitachi Perkin-Elmer VIS-UV spectrophotometer using matched 1-cm pathlength quartz cuvettes. Sample fluorescence was measured using a model 110 Turner fluorometer equipped with a combination of narrow pass filters (numbers 811, 816+831) selected for excitation at 360 nm and emission at 460 nm. The fluorometer was calibrated against a 15 µg liter^{-1} solution of quinine sulphate, which yielded a fluorescence intensity of 30 units at a setting of 10×. Fluorescence and absorbance values were determined against appropriately diluted buffer blanks in all cases.

To complement laboratory findings, water samples were collected from 55 lakes in southwestern Michigan. The samples were filtered through precombusted (525°C), 0.5-µm pore size Reeve Angel glass fiber filters, and the filtrates were immediately analyzed for pH, fluorescence, and absorbance at 250 and 400 nm. Filtrate subsamples were acidified to pH 2 with HNO_3 and were later analyzed for dissolved calcium using atomic absorption spectrophotometry. In addition, relationships between A_{250}, fluorescence and DOC were examined in a small hardwater lake (Lawrence Lake, Barry County, Michigan).

RESULTS AND DISCUSSION

Elution profiles of DOC, A_{250} and fluorescence of the four materials are presented in Figure 1. The profiles are strikingly similar to those of soil humic acids determined under similar conditions (Söchtig 1975).

If absorbance at any wavelength in unfractionated DOM is to be used

Figure 1. Elution profiles of absorbance, fluorescence and dissolved organic carbon in four humic materials on Sephadex G100. Upper left: Nuphar leachate. Upper right: Typha leachate. Lower left: Typha humic materials. Lower right: Kraft indulin AT lignin. DPM = disintegrations $minute^{-1}$ 250 μl^{-1}.

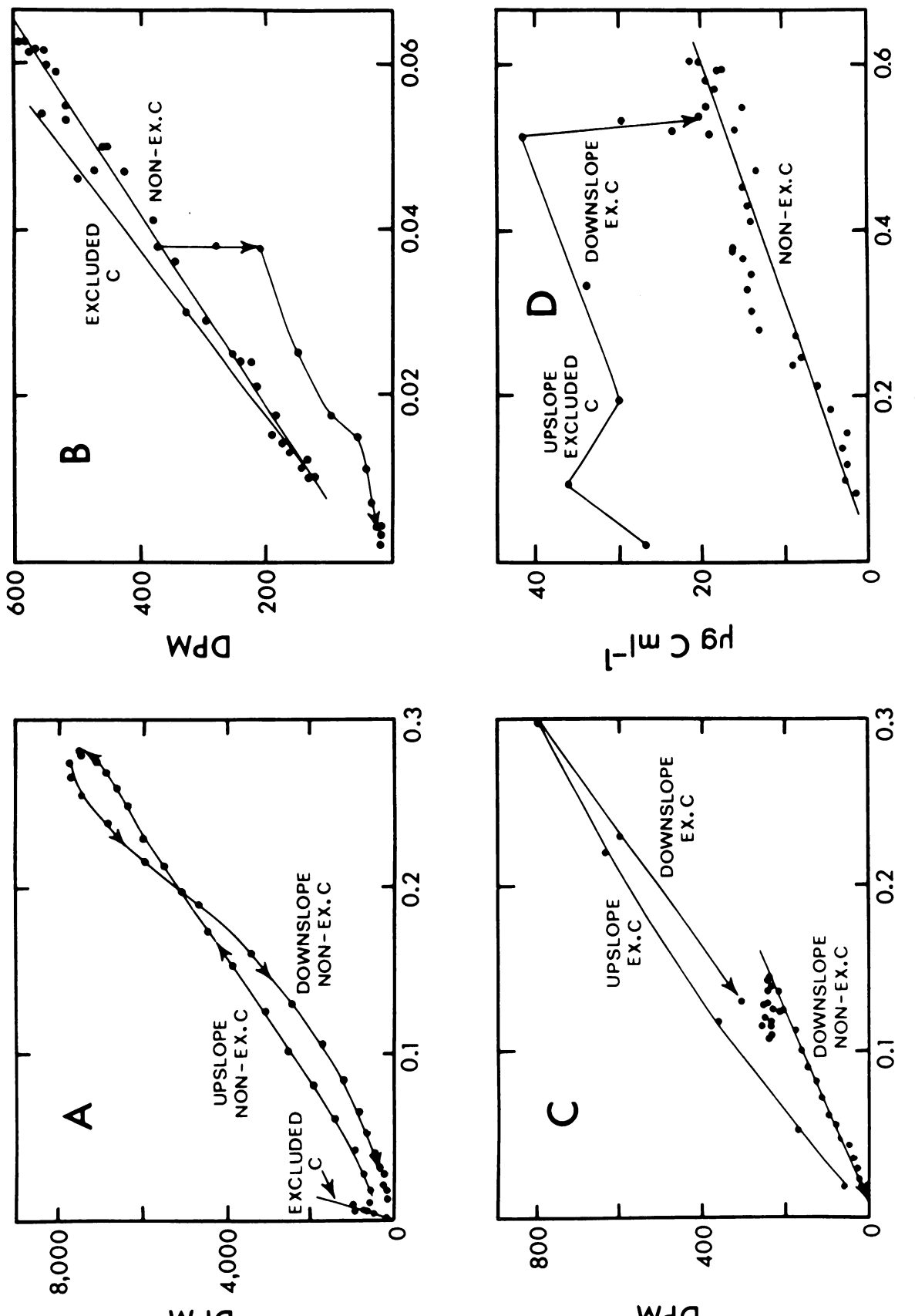
ABSORBANCE (250nm) (——)
FLUORESCENCE (10x) (-·-·-)
DPM (·····)
pg C ml⁻¹ (·····)
Ve/Vt

as a measure of DOC concentration, absorbance (A) must follow Beer's law such that: $C = kA + b$, where k must be both greater than zero and independent of DOC origin and molecular weight. If, in addition, $b = 0$, the relationship simplifies to $C = kA$, and the entire DOC pool can be quantified using absorbance measurements. When high concentrations of non-absorbing DOC are present (i.e. $b \gg 0$), uncertainty will originate from measures of relatively small changes in absorbing DOC against large backgrounds of non-absorbing DOC.

In the four materials used in these experiments, deviations from Beer's law were minimal, and could be observed only when A exceeded about 0.5. To verify whether or not b was equal to zero and k was both greater than zero and constant, plots of absorbance at 250 nm (A_{250}) vs. carbon were constructed for each of the four materials (Figure 2). The plots, in most cases, could be subdivided into three distinct zones: (1) a region encompassing carbon:absorbance ($C:A_{250}$) when emerging DOM was of high apparent molecular weight (excluded C, >100,000 Daltons); (2) a region of DOM of moderate molecular weight (non-excluded, apparent molecular weight < 100,000), and (3) a low apparent molecular weight DOM region, over which concentrations of DOC declined. Since estimation of precise molecular weights of the eluted DOM is subject to considerable error (Cameron et al. 1972), we make only the assumption that an inverse relationship exists between values of V_e/V_t and DOM molecular weight.

In all four cases, the observed $C:A_{250}$ relationships violated to one degree or another the conditions of k independence or b not significantly different from 0. In the case of Kraft lignin, $b \gg 0$, indicating the presence of substantial quantities of high molecular

Figure 2. Absorbance-carbon relationships as a function of DHM apparent molecular weight. Ex. C = excluded carbon (apparent molecular weight > 100,000); non-ex. C = non-excluded carbon (apparent molecular weight < 100,000); upslope = region of increasing carbon concentrations; downslope = region of decreasing carbon concentrations. Arrows designate directions of decreasing apparent molecular weight. A. Nuphar leachate; B. Typha leachate; C. Typha humics; D. Kraft lignin. (DPM = DPM per 250 µl).


A
UPSLOPE NON-EX.C
DOWNSLOPE NON-EX.C
EXCLUDED C
DPM
8,000
4,000
0
0.1
0.2
0.3
B
EXCLUDED C
NON-EX.C
DPM
600
400
200
0.02
0.04
0.06
C
UPSLOPE EX.C
DOWNSLOPE EX.C
DOWNSLOPE NON-EX.C
DPM
800
400
0
0.1
0.2
0.3
D
UPSLOPE EXCLUDED C
DOWNSLOPE EX.C
NON-EX.C
μg C ml⁻¹
40
20
0
0.2
0.4
0.6
ABSORBANCE (250 nm)

weight (>100,000 Daltons), non-absorbing DOM. In N. variegatum leachate, C:A_{250} relationships degenerated into non-linearity on the low molecular weight, decreasing C concentration portion of the plot, while C:A_{250} relationships for I. latifolia humic materials were linear only on the low molecular weight, decreasing C concentration region. In addition, k appeared to be a function both of the apparent molecular weight of the eluted DOM and of material origin (Figure 3), with DOM of lower apparent molecular weight absorbing consistently more per unit carbon than DOM of higher molecular weight.

If we make the dual assumptions that high and low molecular weight absorbing humic materials in the DOM pool are of similar composition and roughly spherical in shape (e.g. that high molecular weight humic substances are complex polymers of smaller humic constituents: Schnitzer and Khan 1972), increases in A_{250} per unit C would be expected in lower molecular weight DOM simply from considerations of molecular geometry. As a first approximation, absorption of photons by spherical DOM molecules will be proportional to molecular cross-sectional area, while carbon content will more closely correspond to molecular volume. In addition, in high molecular weight humic materials, strongly absorbing hydrophobic groups will be spatially restricted to the more central portions of the molecule, where their absorbing characteristics will be at least partially masked by more external, lower-absorbing hydrophilic groups. In the four materials used in these experiments, A_{250}:C increased 2-11 as relative molecular weight of the DOM declined, and is consistent with this hypothesis.

Plots of fluorescence vs. carbon for the four materials demonstrated even more marked asymmetry (Figure 4) than did absorbance,

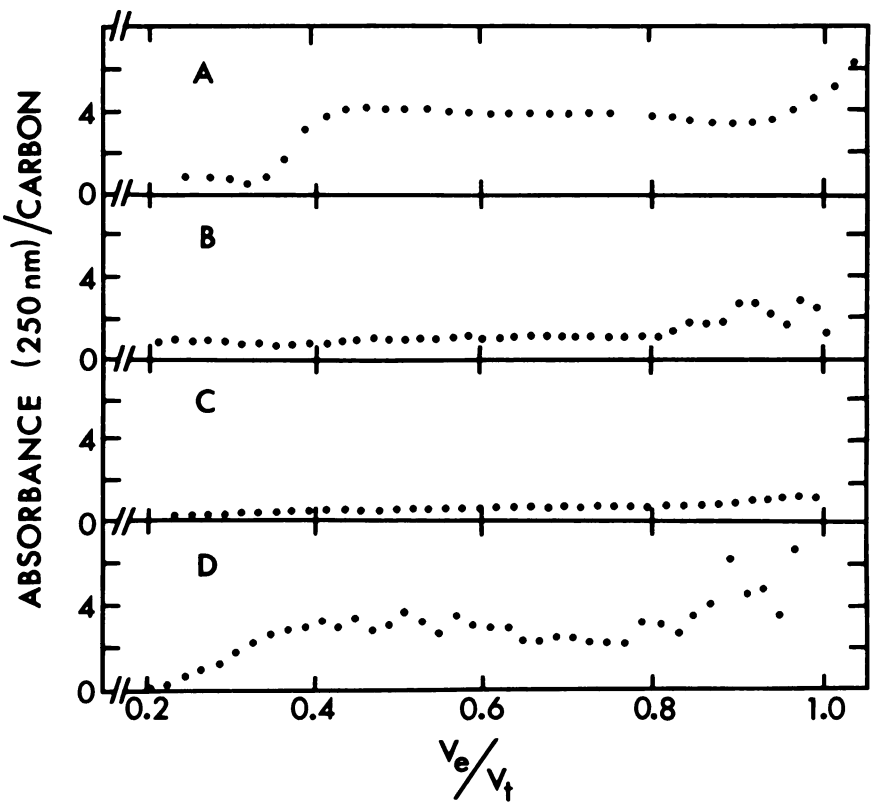
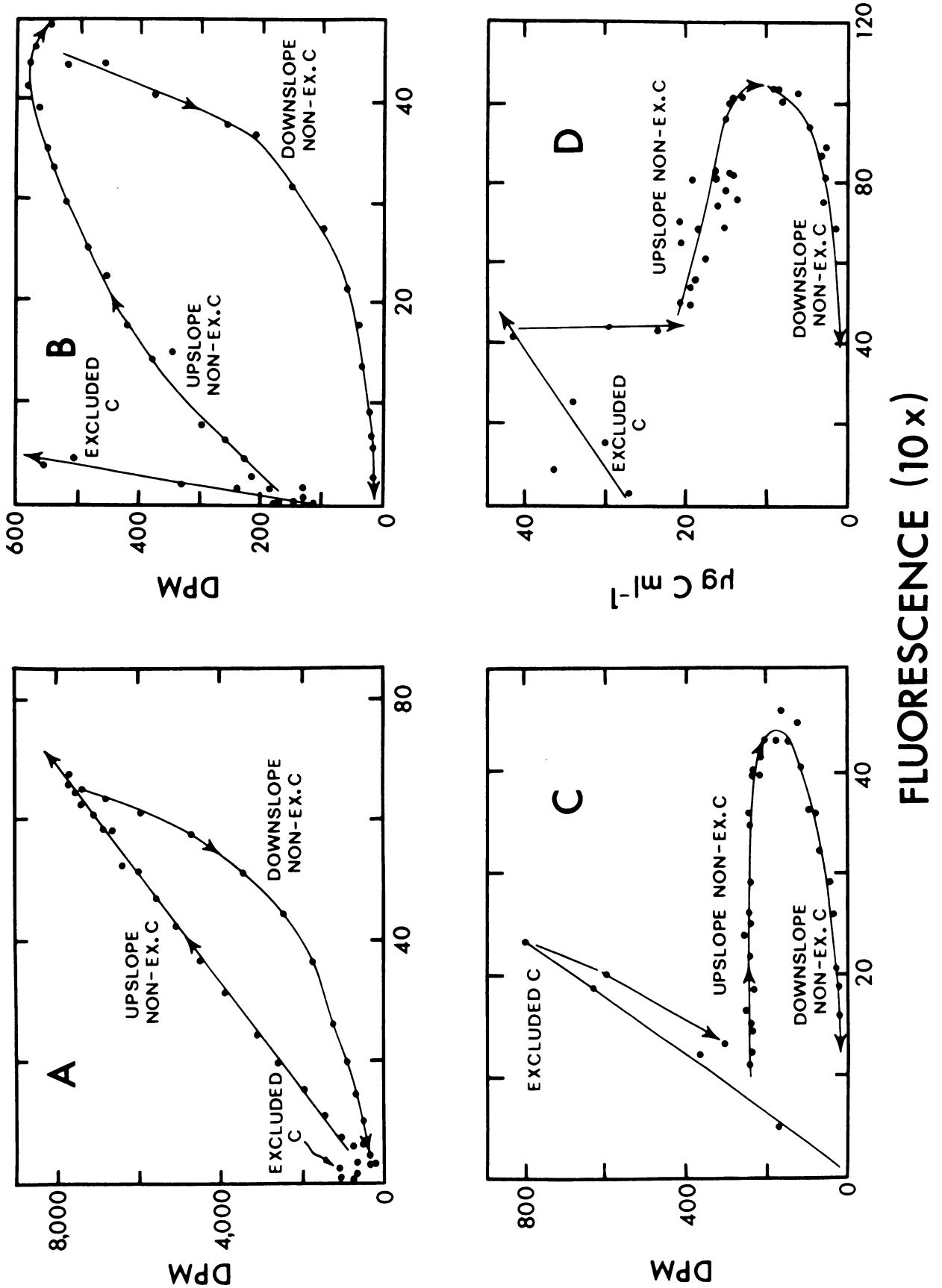


Figure 3. Relative absorbance per unit carbon as a function of DHM apparent molecular weight. A. Nuphar leachate; B. Typha leachate; C. Typha humics; D. Kraft lignin.

Figure 4. Fluorescence-carbon relationships as a function of DHM apparent molecular weight. Ex. C = excluded carbon (apparent molecular weight > 1000,000); non-ex. C = non-excluded carbon (apparent molecular weight < 100,000); upslope = region of increasing carbon concentrations; downslope = region of declining carbon concentrations. Arrows designate directions of decreasing apparent molecular weight. A. *Nuphar* leachate; B. *Typha* leachate; C. *Typha* humics; D. Kraft lignin. (DPM = DHM per 250 µl).


A
B
C
D
EXCLUDED C
UPSLOPE NON-EX.C
DOWNSLOPE NON-EX.C
DPM
μg C ml-1
FLUORESCENCE (10 x)

and N. variegatum leachate was the only substance showing any substantial degree of linearity. In the worst case (T. latifolia humics), 20 units of fluorescence corresponded to 25, 240, 600, or 680 units of carbon, depending upon the value of the DOM apparent molecular weight. Acute asymmetry in carbon-fluorescence (C:f) relationships was associated with a close linkage between fluorescence per unit C and DOM apparent molecular weight. As apparent molecular weight of the DOM decreased, f:C increased very rapidly in all four materials (Figure 5), and contributed disproportionately to fluorescence-absorbance ratios (Stewart and Wetzel 1980).

I propose that decreases in f:C in larger molecular weight DOM is a result of losses in fluorescence emission due to increased levels of self-quenching and internal conversion (Schenk 1973), since larger, more complex DOM molecules possess far more energy states than their smaller counterparts. In addition, it is probable that compositional differences between large and small DOM materials exists, which may further accentuate A_{250}:C and f:C shifts with changes in relative molecular weight (Saiz-Jimenez et al. 1978). In any case, however, it is clear that the optical characteristics of DOM are closely associated with its apparent molecular weight, and that in order to use either absorbance or fluorescence as a predictor of DOC concentration in aquatic systems, some evaluation of DOM molecular weight is required.

When corrected for slight differences in filtrate pH, fluorescence-absorbance ratios of the 55 sites were significantly and positively related to concentrations of dissolved calcium (Figure 6). Although both fluorescence intensity and absorbance declined as concentrations of dissolved calcium increased across the spectrum of lakes, losses in

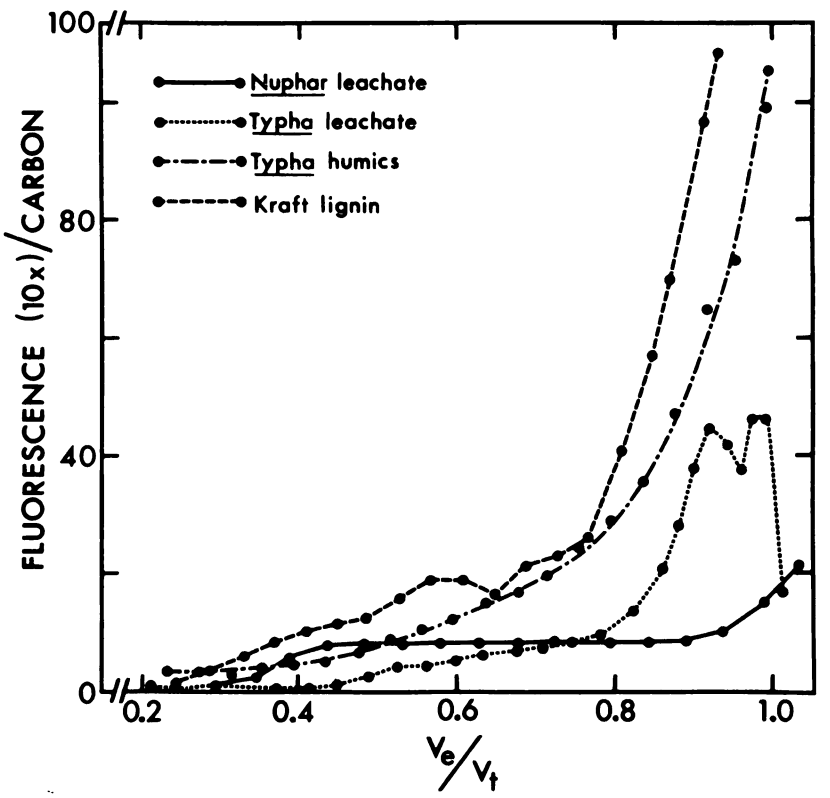


Figure 5. Flourescence (10X) per unit carbon as a function of DHM apparent molecular weight.

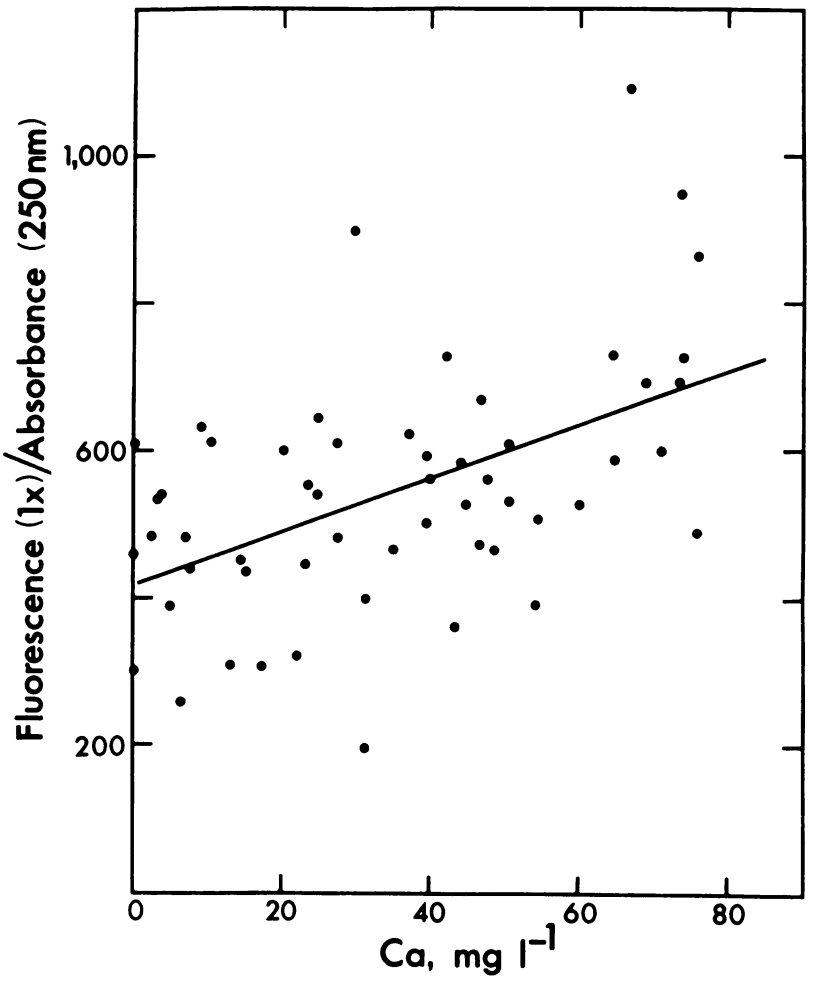


Figure 6. Relationship between concentration of dissolved calcium and the ratio fluorescence (1X)/absorbance (250 nm) of filtered water samples collected from 55 lakes in southwestern Michigan. $P < 0.01$; $Y = 3.62X + 419$; $r = 0.51$.

A_{250} were greater than losses in fluorescence and accounted for most of the increase in f:A_{250}. These data strongly suggest that soft water systems contain relatively larger proportions of high molecular weight DOM than do hardwater systems. Since humic acids are adsorbed to clay (Greenlan 1965; Schnitzer and Kodama 1966; Kodama and Schnitzer 1968) or calcium carbonate particles (Wetzel and Otsuki 1974; Otsuki and Wetzel 1973) in the presence of polyvalent cations, it is likely that hardwater systems are relatively depleted of higher molecular weight humic materials through selective adsorption and precipitation of weakly-fluorescing, strongly absorbing, high molecular weight humic constituents.

The calcium-related fluorescence-absorbance pattern (Figure 6) suggests that no consistent between lake relationship of absorbance and DOC, or of fluorescence intensity and DOC, can be expected because relative molecular weight of DOM between systems decreases as calcium concentration increases, and the optical characteristics of DOM are molecular weight dependent. I conclude that neither fluorescence nor absorbance can be used to predict concentrations of DOC within a system unless it can be clearly demonstrated that mean relative molecular weight of the absorbing and fluorescing DOM remains seasonally and spatially constant (i.e., that f:A_{250} fluctuations are minor), and that absorbing and fluorescing DOM comprises a predominant fraction of the total DOC pool.

Within-system f:A_{250} values from a small hardwater lake were seasonally dependent, and were linked to periods of summer epilimnetic decalcification. This violation invalidates the use of either fluorescence or absorbance to estimate concentrations of DOC in this

electrolyte-rich, hardwater system.

The absorbance-DOC relationships described by Lewis and Tyburczy (1974) and Lewis and Canfield (1977) are likely valid only when applied to soft waters in which system-to-system differences in DOM origin and age are minimized, for any selective process impinging on the molecular weight distribution of DOM (e.g. microbial degradation, photolysis, adsorption and precipitation, inputs of "new" leachate during periods of rainfall, etc.) will alter absorbance-carbon relationships. One must, therefore, use extreme caution when using absorbance to estimate DOC concentrations in most circumstances, and it seems best to avoid the use of fluorescence as a DOC measure in any case.

Finally, lake-to-lake, or seasonal within-lake shifts in molecular weight distribution of dissolved humic materials may be closely coupled to trace metal availability. Other studies have shown that manganese has a strong affinity for low molecular weight DOM (Mantoura and Riley 1975b; Steinberg and Stabel 1978), while, under oxidizing conditions, iron preferentially associates with DOM of high molecular weight (Steinberg and Stabel 1978; Koenings 1976; Koenings and Hooper 1976). The selective removal of high molecular weight DOM in calcium-rich hardwater systems may significantly decrease iron availability, and possibly depress system productivity.

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CHATPER IV

Dissolved humic materials: photodegradation, sediment effects, and reactivity with phosphate and calcium carbonate precipitation

INTRODUCTION

Concentrations of dissolved organic material (DOM) in lakes are often an order of magnitude greater than concentrations of particulate organic matter, and commonly impart a yellowish-brown color to the water. Dissolved organic substances, which can be of either allochthonous or autochthonous origin, are chemically diverse and range from simple compounds (e.g. glycolic acid, glucose, glycine) to recalcitrant and highly polymerized macromolecules formed through partial degradation of plant structural components (e.g. cellulose, hemicellulose, and lignin).

The smaller, more labile constituents of the DOM pool (simple organic acids, sugars, amino acids) can be utilized directly by many heterotrophic organisms. Dissolved humic materials (DHM), by way of contrast, are high molecular weight (200-80,000 daltons) polyheterocondensates consisting of diverse benzenecarboxylic and phenolic acids with esterified n-fatty acids and adsorbed alkanes (Schnitzer, 1978). DHM aggregates are structurally loose and flexible, are easily altered by changes in media pH or ionic strength, and are excellent metal complexation agents (Ghosh and Schnitzer, 1980). The chemical composition of DHM presumably renders it relatively resistant

to further microbial degradation.

Aquatic organisms may utilize DHM either directly (as energy sources, essential growth factors, or non-essential growth substances) or indirectly (via metal complexation) (cf. Saunders, 1957; Shapiro, 1957; Fogg, 1959; Wetzel, 1968; Jørgensen, 1976). At the community level, however, the categories of direct and indirect utilization become obscured, because species interact with not only various DOM constituents, but with each other. For example, if a moderate increase in concentration of a particular DOM fraction has no effect on a given algal species but temporarily relieves bacterial substrate limitation, resulting increases in bacterial numbers may bring the algal and bacterial species into competition for a resource not previously limiting. In this example, the bacterial species clearly utilizes DOM directly; the algal species, however, although not utilizing the DOM either directly or indirectly, can be profoundly influenced by the presence of the material.

Since aquatic bacteria are commonly substrate limited (Kuznetsov, 1970; Rheinheimer, 1974), and because algae can be limited by relatively rare inorganic materials (e.g. phosphorus, iron) whose availabilities may in turn regulated by the types and concentrations of DHM present, DHM is likely to play a central role in pelagial metabolic processes.

In many lakes, DHM originates either allochthonously from surrounding wetland and drainage basin vegetation, or autochthonously from shallow, densely vegetated littoral areas. In either instance, DHM can be exposed to relatively high intensities of light and to large areas of sediment surface. In this paper, we assess the relative

importance of these two factors (photodegradation, exposure to sediment/pH) in regulating DHM quantity and quality. Secondly, considerable data indicate that DHM interferes with the formation of $CaCO_3$, and that co-precipitation of orthophosphate can occur as calcite sediments from the epilimnion of hardwater lakes during summer. In the present investigation, we present data on some relationships between DHM and orthophosphate, and between DHM and $CaCO_3$ precipitation, in order to more clearly delineate modes through which DHM and pelagial microflora are likely to interact.

HUMIC MATERIAL PHOTOLYSIS

Introduction

Gjessing (1970, 1976), Chen et al. (1978) and others have shown that humic materials are photo-oxidized when exposed to UV light. In acid conditions humic materials are photo-oxidized to $CO_2 + H_2O$, while alkaline conditions result in degradation to inorganic carbonates. Photolysis of DHM under some natural conditions may significantly reduce amounts of aquatic humus. However, during exposure to sunlight of an intensity sufficient to cause photolysis of aquatic humus, a series of confounding events occurs: water temperature increases due to the absorption of light, and photodegradation products (CO_2, carbonates) accumulate. Increases of water temperature substantially decrease the solubility of calcite, and humic degradation products alter both water pH and buffering capacity. In addition, losses of humic materials through photolysis may allow $CaCO_3$ precipitation to proceed by removal of threshold quantities of DHM which would normally inhibit the formation of $CaCO_3$ crystals. Interactions between concentrations and quality of humic materials, UV light, pH, water

temperature and the carbonate-bicarbonate equilibrium lead to a nearly intractable matrix of potential outcomes.

Evidence is presented here to show that various humic substances are rapidly altered by UV light, and that sunlight is sufficient to initiate changes in the spectral characteristics of naturally-occurring dissolved humic materials.

Materials and Methods

Photolysis of several dissolved humic materials was accomplished using the mercury-arc lamp (Engelhard Hanovia 679A) apparatus and procedures described by Manny et al. (1971). The humic materials used in these experiments consisted either of naturally-occurring absorbing and fluorescing organic materials found in lake water samples or of humic materials collected from the inlet of Lawrence Lake (Sec. 27, T.1N., R.9W., Barry Co., Michigan). Additional UV photolysis experiments were conducted using metal-free fulvic acid and humic acids obtained from aged Typha leachate (cf. Stewart and Wetzel, 1980b). Concentrated humic materials were quantitatively dissolved in ultrapure Millipore "Q" water to final concentrations of 4.5-16 mg $liter^{-1}$ and were adjusted to pH 8.40 with NaOH, while lake water samples were photooxidized without pH adjustment after glass fiber filtration (0.5-μm pore size, Reeve Angel 984-H). Subsamples were withdrawn from the combustion chamber at 1.0-minute intervals using a 50-ml syringe equipped with a 40-cm Teflon (1.5 mm dia) tubing extension. To preclude pH changes from confounding interpretation of fluorescence and absorbance measurements, 5.0-ml subsamples of each time-series experiment were buffered with 0.5 ml Tris (0.05 M, pH 9.0) prior to spectrophotometric analyses. Fluorescence of the buffered samples was

measured with a Turner model 110 fluorometer at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Sample absorbance (250 nm) was determined against appropriate buffer blanks in matched 1-cm pathlength quartz cuvettes with a Hitachi Perkin-Elmer VIS-UV spectrophotometer.

In several experiments, samples of humic materials were incubated in either full sunlight or in darkness to determine susceptibility of aquatic humus to photolysis under natural conditions.

Results and Discussion

UV light generated by the mercury-arc lamp was sufficient to cause substantial losses in sample absorbance and fluorescence in most humic materials within a few minutes (Figures 1 and 2). Estimated times to degrade absorbing and fluorescing humic constituents to 50 percent of initial values ranged from 2-95 minutes (Table 1), depending upon the material and the spectral parameter.

Humic materials derived from Typha leachate (>3,500 daltons) displayed a fluorescent component that was markedly susceptible to UV light, and a second fluorescing component that was much less labile. Metal-free Contech fulvic acid was partially degraded to form additional fluorescing materials during the first 6 minutes of irradiation, and a decrease in fluorescence was found after this interval. Fluorescence of water samples collected from the hypolimnion of Lawrence Lake (19 October 1979) declined at a rate of roughly 50 percent in 4 minutes after an initial lag time of 1-1.5 minutes, and its photodegradation was essentially complete within 16 minutes.

In all samples, absorbance at 250 nm declined more slowly than did fluorescence over the first 2-3 minutes. Rates of loss of absorbance

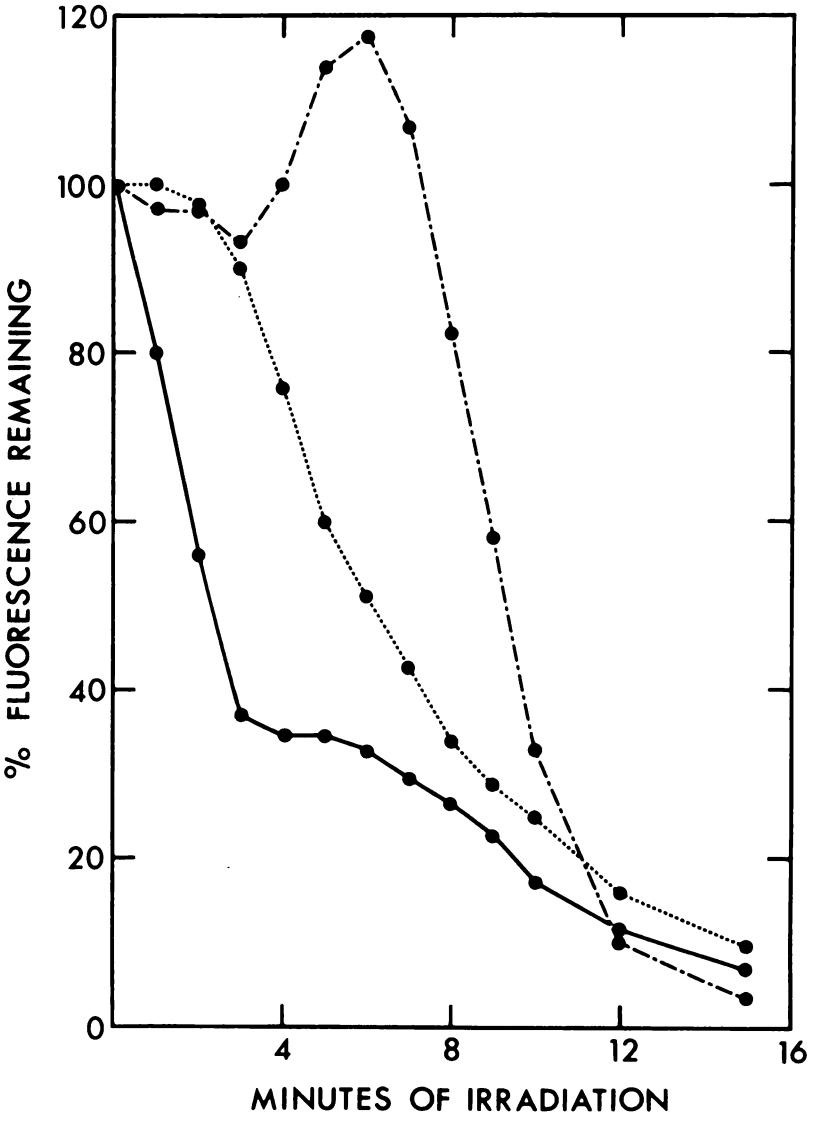

Figure 1. Percent of fluorescence (excitation at 360, emission at 460 nm) remaining during exposure to high-intensity UV light. (——— = Typha leachate); (—·—·— = Contech fulvic acid); (····· = hypolimnetic water sample, Lawrence Lake).

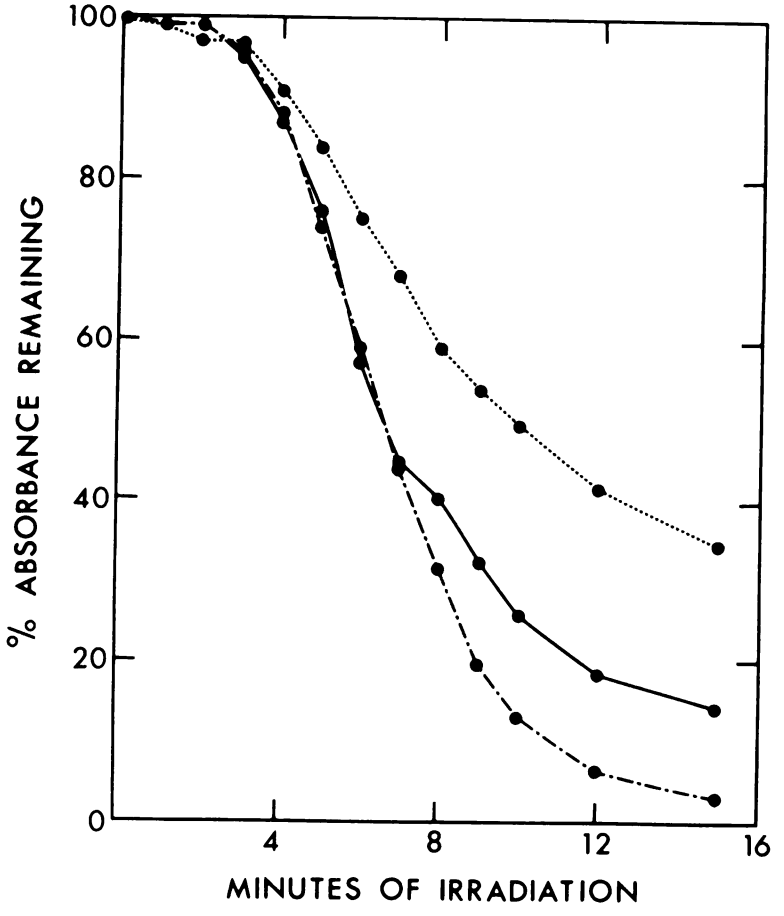

Figure 2. Percent of absorbance (250 nm, 1 cm path-length cells) remaining during exposure to high-intensity UV light. (——— = Typha leachate); (— — — = Contech fulvic acid); (····· = hypolimnetic water sample, Lawrence Lake).

Table 1. Time (min) required to completely degrade absorbing (250 nm) and fluorescing (360 ex., 460 em.) components of selected humic materials.

Humic source	Absorbing component	Fluorescing component
Purified fulvic acid (Contech)	18	18
Lawrence Lake inlet aquatic humus	19-20	18
Typha humic acid (>3,500 daltons)	>25	17-25
Lawrence Lake hypolimnetic water	>35	22-24
Typha humic materials, degraded for 1.5 yr.	>>300	>>300

typically increased thereafter, but rates of absorbance loss were consistently less than rates of loss of fluorescence. In water samples collected from the hypolimnion of Lawrence Lake, loss of absorbance was much slower than rates of loss of fluorescence, suggesting that, in that environment, absorbing materials were more resistant to photolysis. In this instance, irradiation with UV light led to an increase in the fluorescence-absorbance ratio (Stewart and Wetzel, 1980a) through time. In Lawrence Lake, the dissolved organic nitrogen component was UV-reductive to NO_3-N rather than to NO_2-N (Manny et al., 1971). Consequently, the slow rate at which absorbance decreased during photolysis was unlikely the result of composite measures of increases in NO_2-N and decreases in UV-absorbing humic materials.

In similar experiments, 8-12 minutes of UV irradiation were sufficient to reduce the fluorescence(3X)-absorbance ratio of filtered Lawrence Lake inlet water from 556 to 210-270, and 15 minutes of exposure to the UV light source lowered the ratio to 180. Consideration of fluorescence-absorbance ratios as an index of relative molecular weight leads to the suggestion that, in these experiments, photolysis of lower molecular weight humic materials proceeded rapidly compared to high molecular weight humic substances. In Lawrence Lake, summer epilimnetic fluorescence-absorbance ratios approach 180, and decrease to this value from values of 300-350 at the time of ice-loss in the spring (Stewart and Wetzel, 1980b). The UV-induced decline in fluorescence-absorbance ratios of inlet water suggests that *in situ* declines in fluorescence of epilimnetic water may at least partially result from photolysis of UV-susceptible low molecular weight humic constituents during spring and summer, when high light prevails in the

surface waters.

To explore this possibility, 100 ml of Millipore "Q" water was amended with 13.5 mg of leachate derived from uniformly ^{14}C-labelled water lily (Nuphar variegatum) (Stewart and Wetzel, 1980b), and the pH was adjusted to 8.0 with 1 N NaOH. Aliquots of this solution were incubated in open petri dishes at 18-23°C in full sunlight and in darkness for 5.5 h. During the experiment, replicate 0.5 ml subsamples were withdrawn and were immediately diluted with 4.0 ml "Q" water and 0.5 ml 0.025 M borate buffer (pH 8.6) prior to measurements of fluorescence and absorbance. Other subsamples (0.5 ml) were radioassayed via liquid scintillation (Beckman LS8000) in order to correct for evaporative losses.

In this experiment, fluorescence per unit carbon in the sample exposed to full sunlight declined through time, while absorbance per unit carbon was unaffected (Figure 3). These data are similar to those reported by Kramer (1979), but occurred over much smaller time scales (hours rather than days).

HUMIC MATERIAL-SEDIMENT/pH INTERACTIONS

Introduction

A variety of inorganic soil components serve as oxidation catalysts that accelerate the polymerization of phenolic compounds into DHM (Wang and Li, 1977; Wang et al., 1980). In addition, sediments contain enormous numbers of inorganic and organic binding sites, the reactivity of which are regulated by pH, ionic strength, and activity coefficients of the various organic and inorganic species in solution. Likewise, the macromolecular structure of DHM is dependent upon media pH and ionic strength (Ghosh and Schnitzer, 1980). Consequently, as

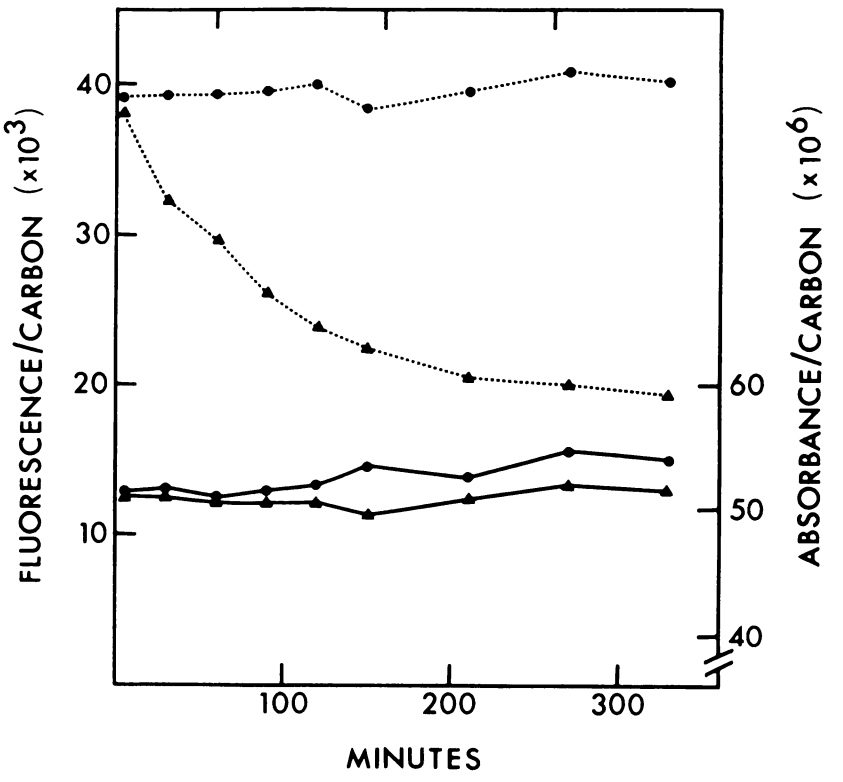

Figure 3. Loss of fluorescence (----------) and absorbance (——————) of ^{14}C-labelled Nuphar variegatum leachate exposed to full sunlight (▲) or incubated in darkness (●).

dissolved organic materials released from decomposing plant tissues interact with sediment material, DHM can be formed and chemically altered. Reactive DHM may bind to sediment particles, while labile DOM and DHM may be consumed by extensive heterotrophic activity associated with organic-rich sediments. Although the extent of alteration of DOM and DHM by lake sediment and associated microflora is currently unknown, data presented in the following experiments indicate that sediment type and pH are important abiotic regulators of DHM.

Materials and Methods

Organic-rich sediments were collected from the inlet of Lawrence Lake and sieved through a Tyler 10-mesh screen (1.91 mm) to remove larger organic debris and were collected on a Tyler 35-mesh screen (0.45 mm). The sediment material so collected was resuspended 5 times in fresh Millipore "Q" water and was allowed to drain thoroughly before use. Purdy Bog (Sec. 36, T.1N., R.9W., Barry Co., Michigan) sediment material contained large amounts of unconsolidated Sphagnum, and it was necessary to homogenize this material (2.0 minutes, Waring blender) prior to sieving and washing.

100-ml aliquots of freshly-collected, filtered (0.5-μm pore size, Reeve Angel 984-H) Purdy Bog lagg water was dispensed into each of 54 flasks. 30 flasks were each amended with 12.3 g of washed Lawrence Lake sediment material, and the remaining 24 flasks were each amended with 12.3 g of homogenized, washed Purdy Bog sediment material. In each experiment, pH values of replicate flasks were adjusted with either NaOH or HCl (both at 1.0 N). In the experiment using Lawrence Lake sediment material, flask contents were additionally amended with 0-2 ml $CaCl_2$ (200 mM) to final concentrations of 0, 1, 2, 3, or 4 mM Ca

at each pH to determine relative importance of ionic strength. $CaCl_2$ amendments were not used in experiments utilizing Purdy Bog sediment material.

After 24 h, 8-10-ml subsamples from each flask were filtered (0.5- m pore size, Reeve Angel 984-H) to remove sediment material. Replicate 1.0-ml volumes were diluted in 4.0 ml "Q" water buffered to pH 9.45 with 0.5 ml borate-NaOH buffer (0.025 M) prior to spectrophotometric analyses of fluorescence (excitation at 360 nm, emission at 460 nm) and absorbance (250 nm, 1-cm quartz cuvettes). pH values of all flasks were measured to the nearest 0.01 unit both at the beginning and end of the experiments. Fluorescence(1X)-absorbance ratios (f:A) were calculated for each sample as described by Stewart and Wetzel (1980a).

Results and Discussion

Figures 4 and 5 demonstrate that sediment type and pH both markedly influenced the nature of the DHM used in these experiments. In the experiment using Lawrence Lake sediment material, f:A values were low (50-70) at low pH values, and increased rapidly to 135-150 as pH increased to 6.5 (Fig. 4). At pH values greater than 6.5, however, filtrate f:A again declined. Increasing $CaCl_2$ concentrations depressed f:A at pH 6, but had no significant effects under more alkaline or acid conditions (Table 2). By way of contrast, filtrate f:A in flasks amended with Purdy Bog sediment material declined almost uniformly as pH increased (Fig. 5). Lawrence Lake sediment material released absorbing organic material when pH values were less than 4.65 or greater than 8.0, and exhibited a net uptake of absorbing material over a pH range of 4.65-8.0 (Figure 6). Fluorescence of DOM was reduced by

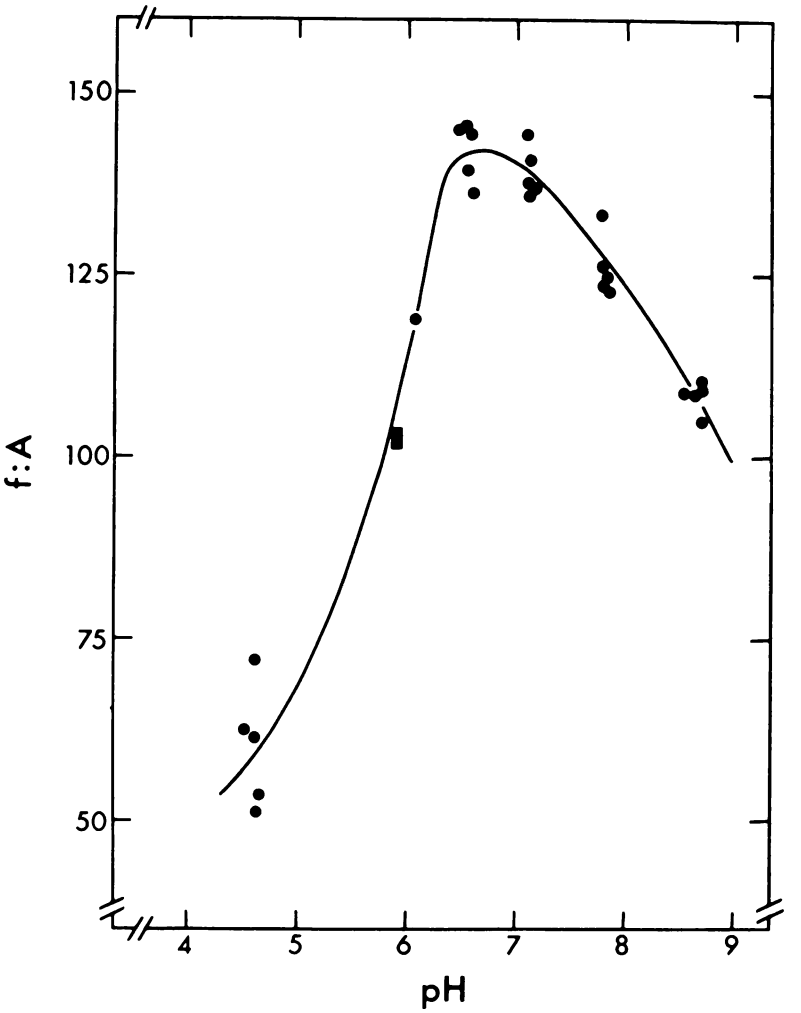

Figure 4. Fluorescence-absorbance ratios of Purdy Bog lagg water exposed to pH-adjusted Lawrence Lake inlet sediment (see text for details). Each point represents the mean of triplicate determinations. The rectangle represents the range of initial f:A-pH measurements.

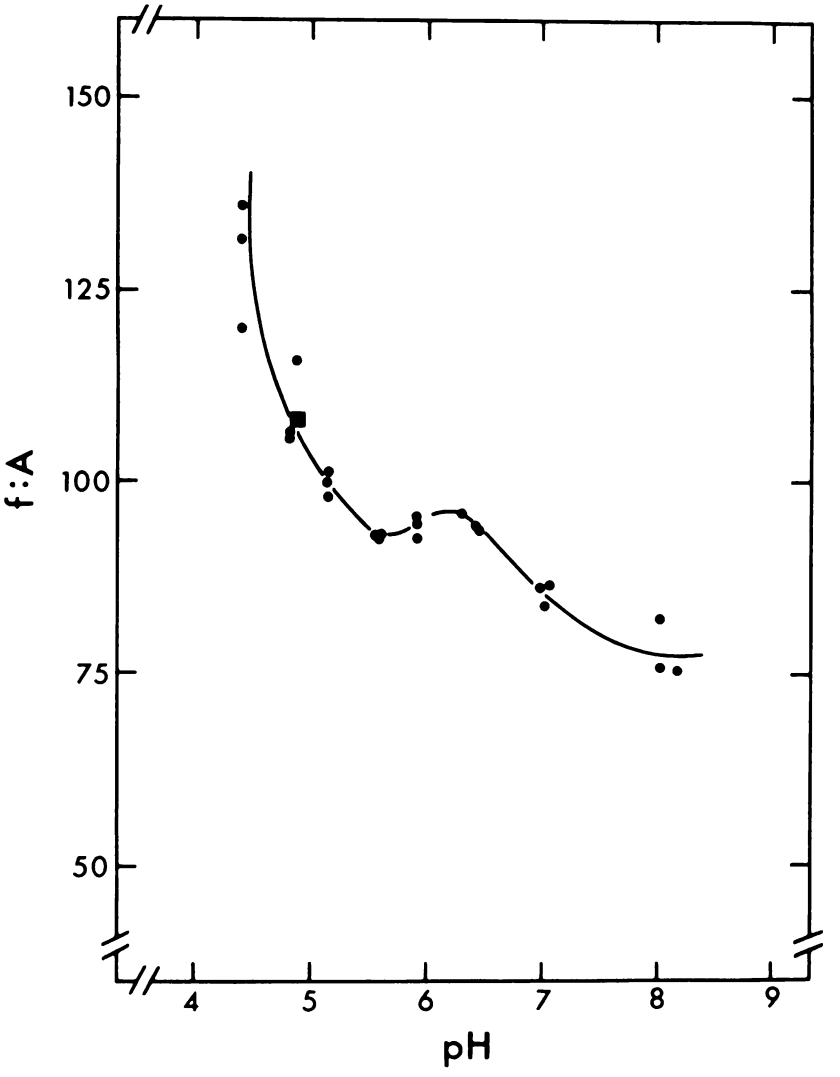

Figure 5. Fluorescence-absorbance ratios of Purdy Bog lagg water exposed to pH-adjusted Purdy Bog sediment (see text for details). Each point represents the mean of triplicate determinations. The rectangle represents the range of initial f:A-pH measurements.

Table 2. Effect of $CaCl_2$ on filtrate fluorescence (1X): absorbance values in mixtures of Purdy Bog lagg water and Lawrence Lake sediment at pH 6.0. (See text for details).

$CaCl_2$ added, mM	fluorescence-absorbance ratio*
0	119
1	105
2	99
3	89
4	86

* Each value represents the mean of three replicates.

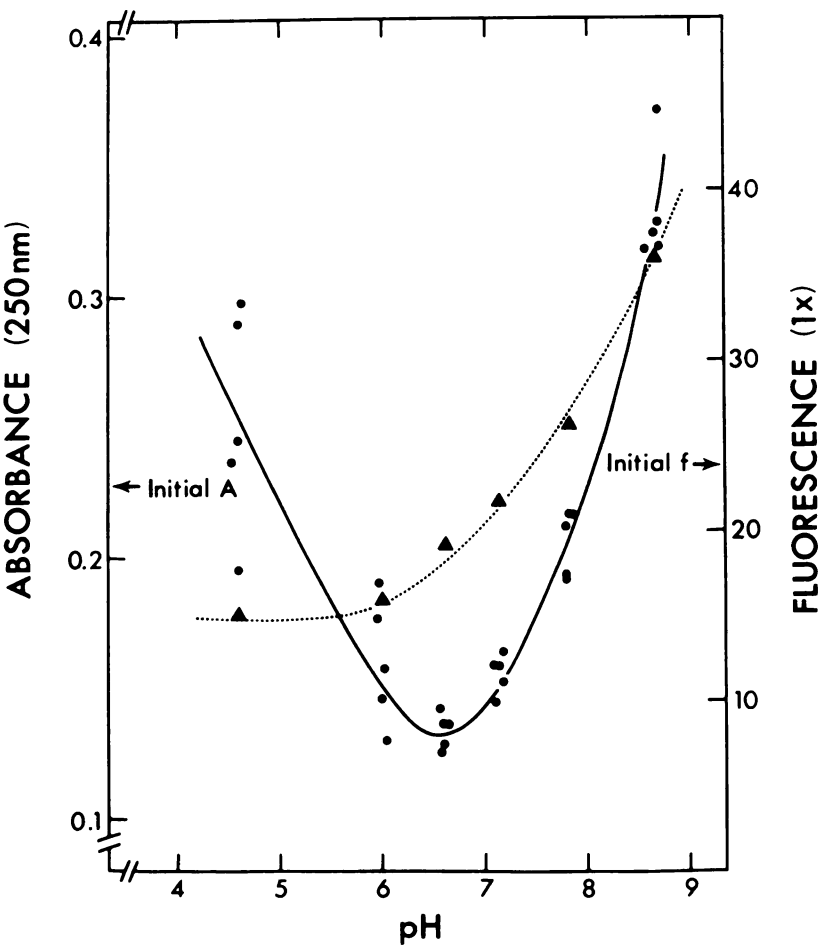

Figure 6. Absorbance (solid line) and fluorescence (dotted line) of Purdy bog lagg water exposed to Lawrence Lake inlet sediment. Fluorescence values (▲) represent means of 15 measurements. Absorbance values (●) represent means of triplicate measurements.

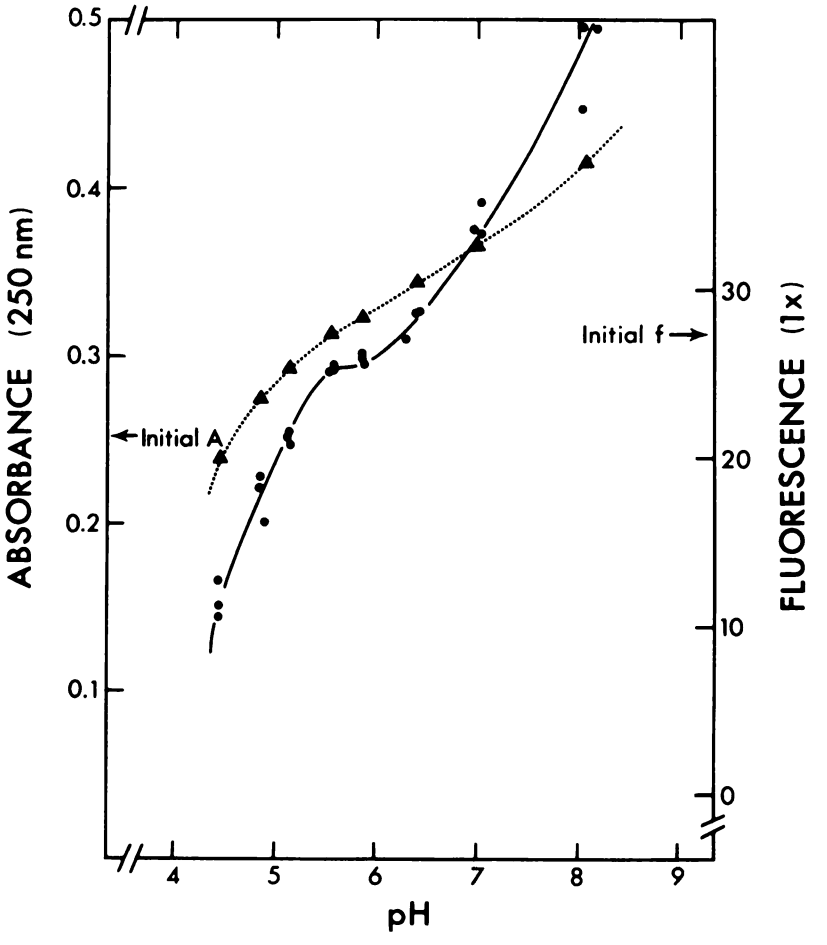

Figure 7. Absorbance (solid line) and fluorescence (dotted line) of Purdy Bog lagg water exposed to Purdy Bog sediment. Fluorescence values (▲) represent means of 15 measurements. Absorbance values (●) represent means of triplicate measurements.

Lawrence Lake sediment material when the pH was below 6.6 and was released from the sediment when conditions were more alkaline.

Purdy Bog sediment material exhibited a net release of absorbing material when pH exceeded 5.15, and a net release of fluorescence above pH 5.6 (Fig. 7). At lower pH values, this sediment adsorbed both fluorescing and absorbing DHM.

The data suggest that sediment type and pH strongly influence quantity and quality of DHM. Since absorbance and fluorescence characteristics of DHM overlap (Stewart and Wetzel, 1980b), my data do not allow a more precise interpretation of the role of pH on sediment uptake of DHM. It seems likely, in addition, that pH-sediment-DHM interactions will depend upon characteristics of the DHM, so that extrapolation from these data becomes even more difficult. However, leachate released from senescent Typha latifolia and Carex sp. during rainstorms had f(3X):A values of 80-100, while water entering Lawrence Lake through the main inlet during rainstorms typically had f(3X):A values around 500, indicating that sediment underlying emergent vegetation near the Lawrence Lake inlet selectively removes higher molecular weight DHM and slowly exports lower molecular weight DHM between rain events.

HUMIC MATERIAL-ORTHOPHOSPHATE INTERACTIONS

Introduction

Under conditions of low pH and low redox potential, dissolved humic materials may associate with orthophosphate in the presence of iron (cf. Blazka, 1979; Francko and Heath, 1979) and render it inaccessable to phytoplankton. Since concentrations of DHM can often be two or three orders of magnitude greater than concentrations of

orthophosphate, even low binding affinities between these two materials may place substantial constraints upon available phosphate. The potential interaction between orthophosphate and DHM should be taken into account when attempting to partition direct and indirect relationships between DHM and pelagial algae, because phosphate limitation in lakes is common.

Estimates of binding can be made using a form of zonal analysis with gel permeation chromatography (Mantoura and Riley, 1975). However, humic materials are largely excluded on Sephadex G-25 due to their large apparent molecular weight, while smaller molecules (e.g. orthophosphate) do not elute in the void volume. This characteristic allows use of a simplified means of determining the absence of DHM-orthophosphate binding. Although co-chromatographic elution of orthophosphate and DHM does not constitute proof of DHM-phosphate interaction, failure to demonstrate co-chromatographic elution profiles of orthophosphate and DHM strongly suggests the absence of direct interaction.

Materials and Methods

Sephadex G-25/150 prepared as per manufacturer's instructions was used in both experiments. The chromatographic column had a total volume (V_t) of 22.7 cm^3 (0.95 x 32 cm). The void volume of the column (V_o, 11.3 cm^3) was determined using blue dextran 2000. A flow rate of 1.89 cm^3 cm^{-2} $minute^{-1}$ yielded 0.67-ml fractions every 30 seconds.

In the first experiment, a borate-HCl buffer (0.025 M, pH 8.10) was used to elute a buffer-equilibrated mixture of high molecular weight (>3,500 daltons) ^{14}C-labelled Typha DHM and ^{32}P-orthophosphate. In the second experiment, a mixture of Lawrence Lake inlet DHM and

^{32}P-orthophosphate was chromatographed using filtered (0.5-μm pore size, 984-H Reeve Angel) Lawrence Lake water (pH 8.45) as the eluant. The DHM-phosphate mixtures were incubated at room temperature for 30 minutes prior to chromatography to insure chemical equilibrium.

Subsamples (100 μl) of each eluted fraction were added to 10 ml of scintillation media (Instagel), and were radioassayed for ^{32}P with a liquid scintillation counter (Beckman LS8000). In the first experiment, simultaneous determination of ^{14}C was accomplished by correcting for ^{32}P spillover into the lower-energy ^{14}C window.

Other subsamples (100 μl) of each fraction were diluted with 4.5 ml "Q" water prior to spectrophometric assays of fluorescence and absorbance. Absorbance at 250 nm was determined with a Hitachi Perkin-Elmer VIS-UV spectrophotometer using matched 1-cm pathlength quartz cuvettes. Sample fluorescence was measured using a model 110 Turner fluorometer equipped with a combination of narrow pass filters (numbers 811, 816+831) selected for excitation at 360 nm and emission at 460 nm. The fluorometer was calibrated against a 15 μg liter^{-1} solution of quinine sulphate, which yielded a fluorescence intensity of 30 units at a setting of 10x. Fluorescence and absorbance values were determined relative to appropriately diluted buffer blanks in all cases.

Results and Discussion

Elution profiles for the two experiments are shown in Figures 8 and 9. In both experiments, orthophosphate emerged as a distinct peak over a V_e/V_t range of 0.60-0.90, while the humic materials consistently chromatographed in the void volume (V_e/V_t = 0.45-0.55). Since absorbing and fluorescing elution tails of humic materials

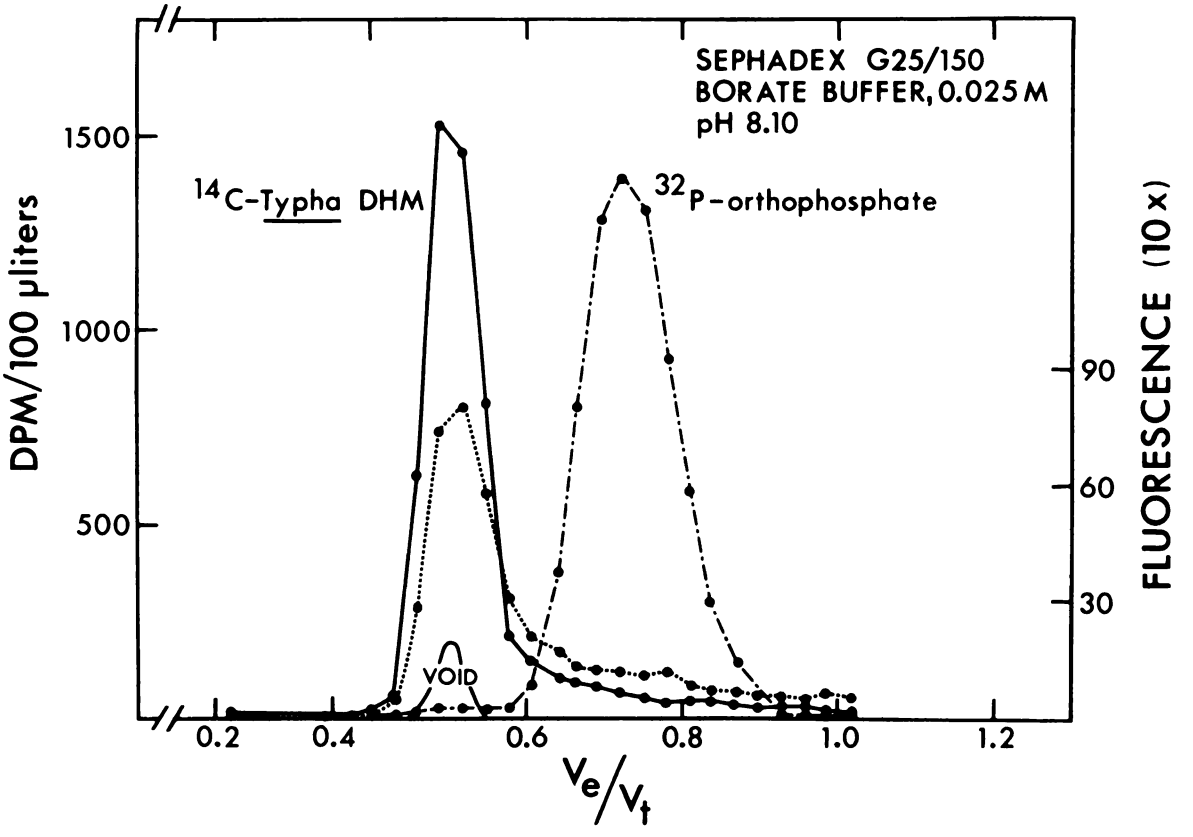

Figure 8. Gel permeation chromatography of mixtures of ^{14}C-labelled Typha DHM and ^{32}P-orthophosphate. (See text for details).

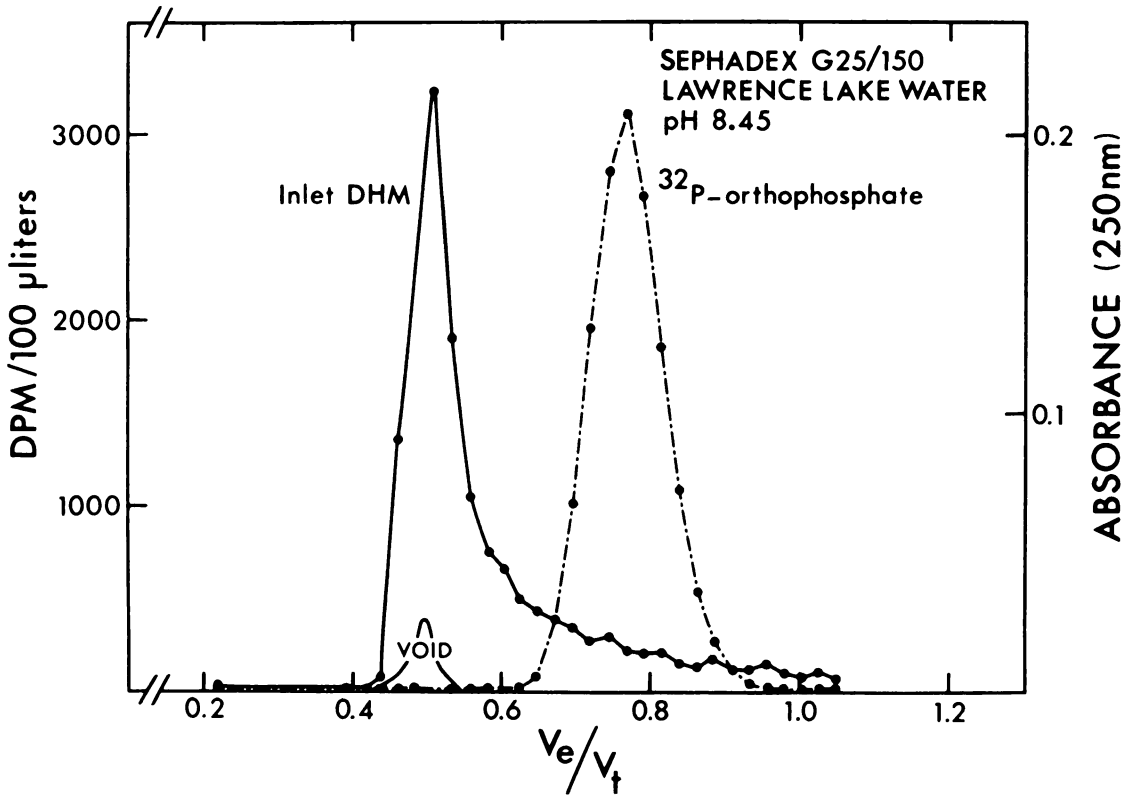

Figure 9. Gel permeation chromatography of mixtures of Inlet DHM and ^{32}P-orthophosphate. (See text for details).

contain disproportionately small amounts of carbon (Stewart and Wetzel, 1980b), binding of orthophosphate to the aquatic humic materials used in these experiments was clearly unimportant.

Calcium can inhibit labile humic acid-metal complexation processes (O'Shea and Mancy, 1978), and may be expected to interfere with orthophosphate binding processes as well. In addition, iron preferentially binds to higher molecular weight humic constituents (Koenings, 1976; Koenings and Hooper, 1976), and selective loss of high molecular weight dissolved humic material occurs as concentrations of dissolved calcium increase (Stewart and Wetzel, 1980b). In the epilimnion of Lawrence Lake, there are low levels of iron, high concentrations of dissolved calcium and a relative depletion of high molecular weight humic material. These factors, in combination with an alkaline pH, aparently prevent regulation of orthophosphate pool by direct binding of free phosphate to dissolved humic materials.

In summary, both theoretical considerations and the failure to demonstrate co-chromatographic elution of orthophosphate and DHM in these experiments strongly suggest that in the epilimnion of Lawrence Lake, binding of orthophosphate to humic materials does not occur. Increases in activity of the enzyme alkaline phosphatase by the microflora of Lawrence Lake in response to low concentrations of naturally-occurring humic materials must be due instead to a more indirect relationship between biota, phosphorus and dissolved humic materials (Stewart and Wetzel, in prep.).

DHM-$CaCO_3$ RELATIONSHIPS

Introduction

In hardwater lakes, epilimnetic decalcification proceeds vigorously during the summer months, for the precipitation of $CaCO_3$ is enhanced by increases both in water temperature and in photosynthetic activities of pelagial microflora (Wetzel, 1975; Kelts and Hsu, 1978). As epilimnetic decalcification proceeds, phosphate and dissolved organic matter are lost from the epilimnion via co-precipitation (Otsuki and Wetzel, 1973; Wetzel and Otsuki, 1974; Rossknecht, 1980). Consequently, processes or mechanisms that retard epilimnetic decalcification should conserve epilimnetic phosphate, and must therefore be of major importance to phosphate-limited pelagial primary producers.

Many substances (e.g. organophosphate compounds, albumin, stearic acid, soluble humic materials) retard the formation of $CaCO_3$ crystals by blocking spiral dislocation growth sites, which forces the crystals to grow by a much slower surface nucleation process (Reynolds, 1978; Reddy, 1978). Potential losses of phosphate through co-precipitation with $CaCO_3$, and the inhibition of $CaCO_3$ precipitation by dissolved humic materials clearly suggest that humic materials may interact with pelagial algae in hardwater lakes through a limiting phosphorus mode, with $CaCO_3$ precipitation an important but indirect mediation process.

Experiments demonstrating inhibition of $CaCO_3$ precipitation by humic materials (Reddy, 1978; Reynolds, 1978), or co-precipitation of organic materials (Otsuki and Wetzel, 1973) often fail to adequately represent in situ conditions, for they utilize uniform seed $CaCO_3$ crystals of great purity, or induce precipitation from

particle-free solution. Co-precipitation of various organic materials is a function of rate of $CaCO_3$ crystal growth, for a slowly growing $CaCO_3$ particle can incorporate organic molecules by surface adsorption. Consequently, artificially low estimates of rates of co-precipitation would be expected by inducing high rates of $CaCO_3$ particle formation. In the following experiment, inhibition of $CaCO_3$ precipitation by fulvic acid was determined under more natural conditions.

Materials and Methods

Inhibition of $CaCO_3$ precipitation by metal-free fulvic acid (Contech; molecular weight 643-951 daltons) was determined as follows: A 1-liter water sample collected from Lawrence Lake (2 m, 16 September 1979) was filtered through Nitex (160-μm mesh-size) to remove large zooplankton. 15-ml aliquots of the filtered sample were dispensed into glass scintillation vials.

In the first experiment, the contents of each of 39 vials were amended with 25 μl of fulvic acid-ultrapure Millipore "Q" water solution (pH adjusted to 8.60 with NaOH) to yield final replicated concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 3.0 and 4.0 mg fulvic acid per liter. Three controls were treated with 25 μl of "Q" water. The 39 vials were loosely capped and incubated on a gyratory shaker (100 rpm) in an environmental chamber at 20°C for 3 days at a light intensity of 100 $\mu E\ m^{-2}\ sec^{-1}$ (L:D = 14:10). On the third day of incubation, the contents of each vial were poured into a clean, appropriately labelled vial. 5.0 ml of "Q" water was added to each decanted vial, and 100 μl of concentrated HNO_3 was added to each of the 78 vials. The vials were tightly capped and were sonicated for 5 minutes to fully disperse small clumps of particles. Concentration

of calcium in each vial was determined using atomic absorbtion spectrophotometry. Volume-corrected amounts of precipitated calcium were expressed as percent of the total Ca:

$$\text{percent Ca precipitated} = \left[\frac{\text{deposited Ca}}{\text{deposited Ca + suspended Ca}} \right] \times 100$$

The contents of the remaining 20 vials were amended with either 25 µl "Q" water or 25 µl of fulvic acid solution sufficient to produce a final concentration of 7.2 mg fulvic acid liter^{-1}. The 20 vials were loosely capped and were incubated under the same conditions as those outlined for the first experiment. Relative rates of community carbon assimilation were determined daily by injecting 100 µl of $NaH^{14}CO_3$ (100 µCi ml^{-1}) into duplicate vials for each treatment. The contents of the vials were incubated without caps in the presence of the labelled bicarbonate for four hrs (10:00-14:00). At the end of each incubation period, rapid termination of photosynthesis was accomplished by addition of 250 µl of concentrated HCl per vial. Contents of the vials were frozen, lyophilized to dryness to remove residual inorganic ^{14}C (McKinley et al., 1978), and were resuspended in 0.5 ml "Q" water. The tightly capped vials were then sonicated (5 minutes) to fully disperse small clumps of particles. Photosynthetically fixed ^{14}C was determined by adding 10 ml scintillation media (Instagel) to each vial and radioassying the contents with a liquid scintillation counter (Beckman LS8000). Background corrections were made before converting activity to dpm.

Results and Discussion

The amount of calcium precipitated onto the walls and bottoms of the glass vials was inversely related to the amount of fulvic acid present (Figure 10). Fulvic acid concentrations of greater than 2.0 mg liter^{-1} inhibited all $CaCO_3$ precipitation. Assuming the true molecular weight of the fulvic acid to be 800 daltons, a 2.0 mg liter^{-1} final concentration of fulvic acid is equivalent to a 2.5×10^{-6} molar solution. Using different methods, Reddy (1978) found that a concentration of 10 mg liter^{-1} of humic acid (K and K Laboratories) caused 75 percent inhibition with 11 days of incubation. Inhibition of $CaCO_3$ precipitation by Contech fulvic acid was much more complete (100 percent) at lower concentrations over shorter intervals of time, suggesting that either the higher molecular weight humic acid used in Reddy's experiments was less inhibitory to the $CaCO_3$ precipitation process, or that in this experiment, the more natural conditions (e.g. non-uniform nuclei, photosynthetic removal of $CO_{2(aq)}$) favored the inhibiting effects of fulvic acid on $CaCO_3$ precipitation.

In another experiment, rates of assimilation of ^{14}C-labelled bicarbonate amended with 7.2 mg fulvic acid liter^{-1} (pH 8.6) did not significantly differ from control populations during the first three days of incubation (Figure 11). These results indicate that fulvic acid amendments did not inhibit the photosynthetic uptake of CO_2.

Results of the two experiments indicate that under natural conditions, low concentrations of fulvic acid are sufficient to substantially alter amounts of $CaCO_3$ precipitated by photosynthesis, and suggest that the inhibitory effect of the humic materials acts directly, rather than indirectly, upon the carbonate-bicarbonate-

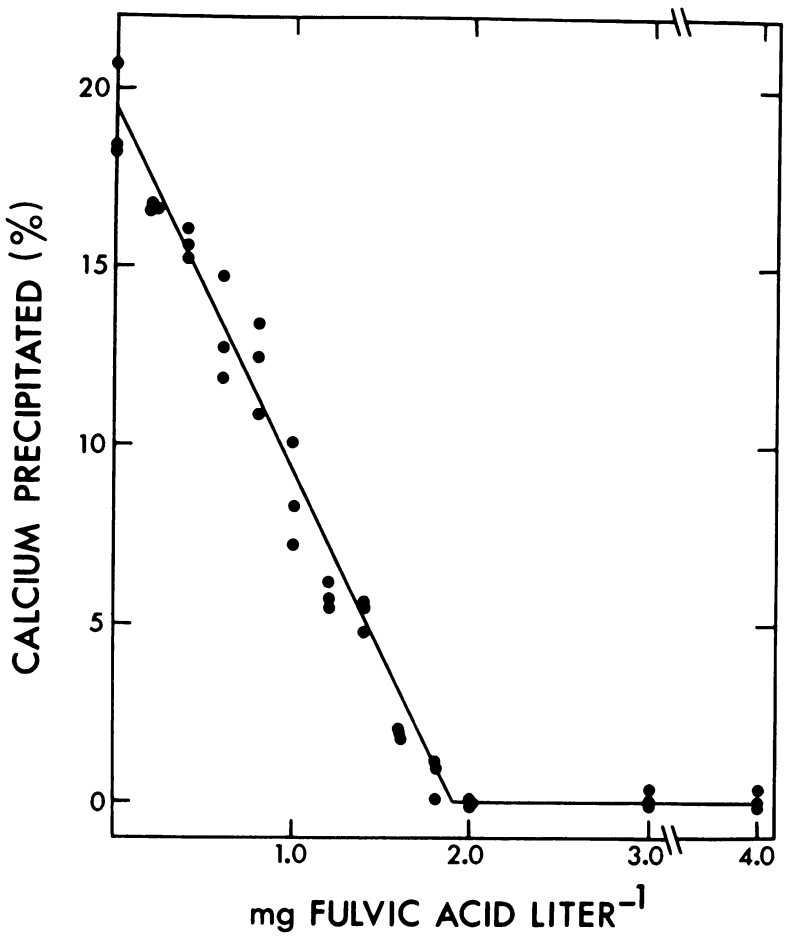

Figure 10. Inhibition of calcium carbonate precipitation by fulvic acid.

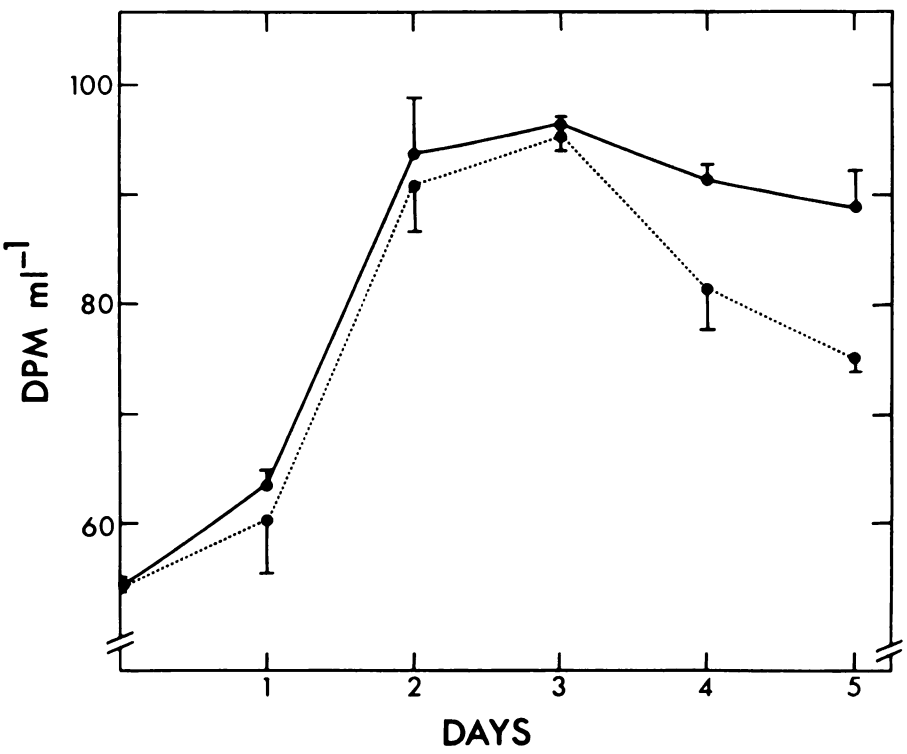

Figure 11. ^{14}C assimilation by natural algal assemblages incubated at 20°C under low light (25 µE m^{-2} sec^{-1}) with (dotted line) or without (solid line) 7.2 mg l^{-1} fulvic acid. Each point represents the mean of triplicate measurements. Error bars designate ± 1 S.E.

CO_2 equilibrium.

During and immediately following intense rainstorms, copious quantities of dissolved humic materials enter the pelagial zone of Lawrence Lake via runoff from the surrounding wetlands and drainage basin (Wetzel, unpublished data; personal observation). Assuming complete runoff from the drainage basin of a surface area seven times that of the lake itself (Wetzel and Otsuki, 1974) and a DOM input concentration of 10 mg liter^{-1}, a 6.5-cm rainfall would result in an additional DOM concentration of ca. 1.5 mg liter^{-1} in a uniformly-mixed 3-m epilimnion. It appears likely that major rain events could fundamentally alter short-term rates of epilimnetic decalcification in Lawrence Lake.

Production of DHM is predominantly a littoral or wetland process, for most of the recalcitrant materials that contribute to DHM originate from partial decomposition of plants that grow either as emergent vegetation in the littoral zone or of more heavily lignified plants that grow in the lake's drainage basin. As the vegetation undergoes scenescence, DHM is produced; during and following intense rains, DHM is rapidly moved from the site of production to the lake. Data presented in this paper indicates that as DHM is moved from the site of production to the epilimnion, losses of DHM occur both to sediment absorption and to photolysis. The two loss processes sequentially raise, then lower the fluorescence-absorbance ratio of DHM (Figure 12), indicating there is an initial selective loss of high molecular weight DHM, followed by a selective loss of DHM of lower molecular weight.

The uniformity of f:A in the inlet to Lawrence Lake during and following rain events indicates that the sediment uptake of high

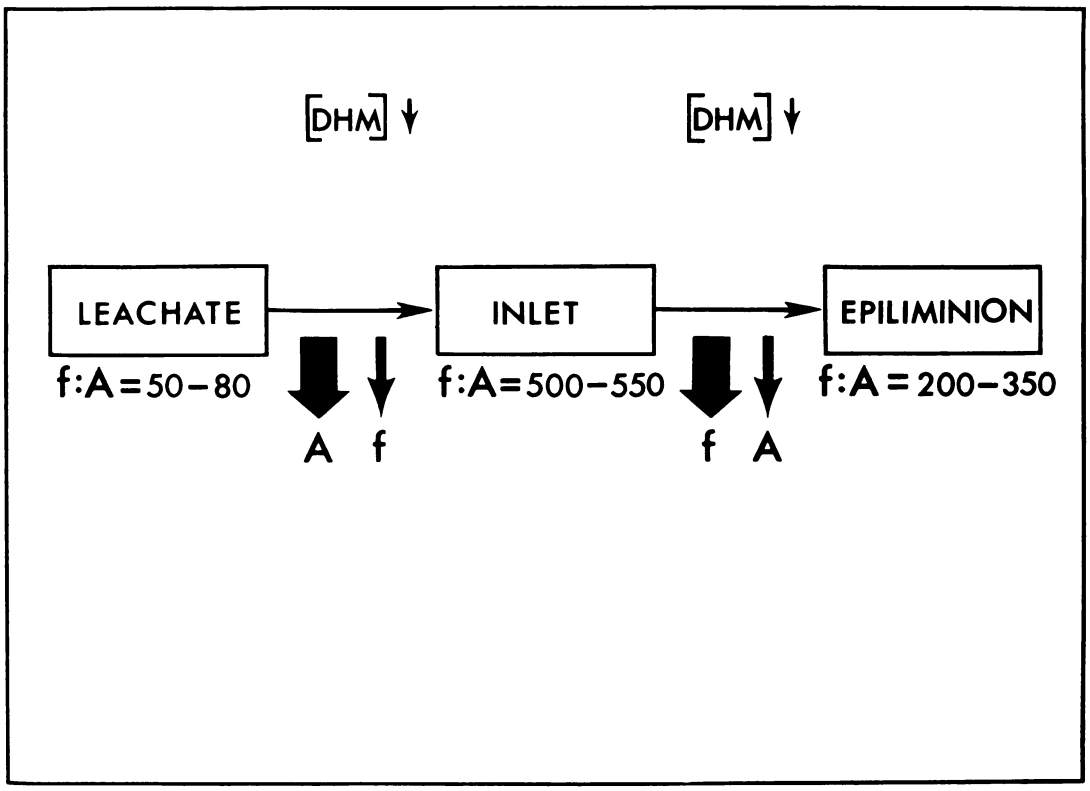

Figure 12. Changes in DHM fluorescence:absorbance ratios as DHM moves from the site of production (leachate) to the epilimnion of Lawrence Lake, Barry County, Michigan. f = fluorescence (3X), a = absorbance (250 nm, 1 cm pathlength) of filtered, buffered water samples (see text for details). Arrow width represents relative loss of sample fluorescence or absorbance as DHM is transported from the site of production to the epilimnion.

molecular weight DHM occurs rapidly. Additionally, since the f:A does not fluctuate markedly during periods of drought, slow microbial degradation of high molecular weight DHM adsorbed to sediment material can occur, with a concomittant, gradual release of DHM of lower molecular weight. In this context, littoral and wetland sediment may act as very effective buffers by closely regulating both the quantity and relative molecular weight spectrum of DHM.

Since assemblages of algae and bacteria from the epilimnion of Lawrence Lake respond differently to high molecular weight DHM than they do to DHM of low molecular weight (cf. Stewart and Wetzel 1981), abiotic regulation of DHM molecular weight may be important in altering patterns of pelagial metabolism. Additional investigations in the area of abiotic regulation of DHM quantity and quality would appear to be both fruitful and urgently required.

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CHAPTER V

Influence of dissolved humic materials on carbon assimilation and alkaline phosphatase activity in natural algal-bacterial assemblages.

INTRODUCTION

Dissolved humic material (DHM) is a conspicuous component of the dissolved organic carbon pool of many freshwater lakes, and is often present in quantities sufficient to impart a brown or yellowish cast to the water. Considerable evidence suggests that DHM can interact with aquatic biota by reducing toxicity or by increasing availability of trace metals (Schnitzer and Skinner 1966, 1967; Martin et al. 1971; Wetzel 1972; Gambel and Schnitzer 1974). Several other studies (Shapiro 1957; McLachlan and Craigie 1964; Prakash and Rashid 1968; Prakash et al. 1973) have shown that humic materials can stimulate or inhibit growth of various algal species in pure culture, while de Haan (1974, 1977) and Strome and Miller (1978) demonstrated that certain humic materials can be utilized for growth by aquatic bacteria. However, although ubiquitously distributed and frequently abundant, little is known about the ecological importance of DHM in pelagial environments. Since bacterial-algal interactions are likely (cf. Fuhs et al. 1972; Rhee 1972; Pearl 1979; Sieburth 1979), it is very unlikely that effects of humic materials on unispecific algal cultures can be safely extrapolated to community level responses.

Algal primary productivity and phosphorus cycling are two pelagial community-level processes that may be influenced by inputs of DHM. In

many lakes, primary productivity is limited by low levels of available phosphorus (Vollenweider 1968; Wetzel 1975), so that overall rates of productivity and phosphorus cycling may be intimately linked. In some cases, productivity may be limited by low concentrations of certain trace metals (cf. Goldman 1960; Sanders 1978), whose availabilities may be altered by DHM. In addition, humic materials are known to interfere with some enzyme systems (Loomis and Battaile 1966), and aquatic microflora commonly prossesses enzymatic means (e.g. acid or alkaline phosphatases) to release orthophosphate from organic phosphorus substrates (cf. Karl and Craven 1981).

In this study, I assess the effects of selected DHM on alkaline phosphatase activity (APA) and on carbon assimilation by naturally-occurring algal-bacterial assemblages in order to obtain more realistic information regarding the ecological significance of DHM in pelagial community processes.

MATERIALS AND METHODS

DHM molecular weight is a major reflection of DHM quality, for low molecular weight DHM is better at metal complexation than DHM of high molecular weight (Rashid 1971; Steinberg and Stabel 1978; Mantoura and Riley 1975), and because DHM of low molecular weight is considered to be more labile than DHM of high molecular weight (Ogura 1975; Strome and Miller 1978). However, in an attempt to assess effects of DHM on community processes, consideration must also be given to DHM origin and concentration. Finally, other experiments indicated potential interactions between light intensity, phosphorus availability and DHM (Stewart and Wetzel 1981). Consequently, the experimental designs outlined below were selected to at least partially cover all of these

considerations.

Algal-bacterial Assemblages

Phytoplankton samples were taken from the epilimnion of Lawrence Lake, Barry County, Michigan immediately before each experiment. The water was filtered through 160 μm mesh-size Nitex to remove large zooplankton, and, depending upon the experiment, 10, 15 or 20-ml samples were dispensed into glass scintillation vials. After addition of humic material and/or phosphate, replicate vials were loosely capped and were incubated at 20°C on a gyratory shaker (100 rpm) placed in an environmental chamber. Light (either 25 or 100 $\mu E\ m^{-2}\ sec^{-1}$) was supplied with Vitalux fluorescent lights on a 14:10 L:D cycle in all experiments. In some cases, samples were filtered through Nuclepore filters of various pore sizes under low vacuum (< 0.5 atm) either prior to humic amendment or at the conclusion of an experiment in order to determine APA or assimilated ^{14}C in different particle-size fractions.

DHM Collection and Molecular Weight Fractionation

Humic materials used in the study were obtained from three sources: ^{14}C-labelled humic acid from decomposed shoots of the cattail Typha latifolia L., dissolved humic materials isolated from the water of a small inlet stream to Lawrence Lake, Barry County, Michigan, and purified, metal-free fulvic acid (Contech, Inc.). The three humic materials were selected to fulfill criteria of generality, specificity and reproducibility, respectively. ^{14}C-labelled Typha humic material (Typha DHM; 8060 dpm mg^{-1}) was collected as described by Stewart and Wetzel (1980b), but was dialyzed (nominal molecular weight (MW) cutoff = 3,500 daltons) against 0.01 N HCl to convert the material from the

ammonium salt to the acid form. Humic materials were isolated from Lawrence Lake inlet water (Inlet DHM) on Amberlight XAD-2 resin as described by Mantoura and Riley (1975), while metal-free fulvic acid (FA; MW = 643-951 daltons) was used without further preparation. Inlet DHM and FA were quantitatively dissolved into 100-ml volumes of Millipore Super "Q" water, and the pH of each solution was adjusted to 8.20 with 1 N NaOH to approximate the pH of the epilimnetic water of Lawrence Lake. The solutions, each containing 3.60 g l^{-1}, were dispensed into ampoules, sealed, and stored at -15°C until use. Typha DHM powder was stored under desiccant, and appropriate amounts of the material were quantitatively dissolved into "Q" water (pH adjusted to 8.20) immediately prior to use.

In some experiments, Typha DHM was fractionated on the basis of apparent molecular weight via gel permeation chromatography (Table 1). Eluted volumes obtained in this manner were appropriately pooled to produce five fractions (I-V) of Typha DHM differing both in apparent molecular weight and in spectral characteristics. The organic material in each of the five fractions was concentrated by lyophilization prior to reconstitution in "Q" water. Subsamples (25-75 µl) of each fraction were radioassayed using liquid scintillation (10 ml Instagel) to determine concentration of dissolved humic carbon. The eluant from another sample of Typha DHM fractionated in the same manner was collected, lyophilized, and reconstituted in "Q" water, and was used as a control DHM source in these experiments.

Phosphate Amendments

In several experiments, the combined effects of phosphate (supplied as KH_2PO_4, usually to a final concentration of 18 µg l^{-1}) and

Table 1. Gel permeation chromatography of ^{14}C-labelled Typha DHM (MW > 3,500 daltons)[1].

Fraction	V_e/V_t range	Fluorescence:Absorbance[2]
I	0.41-0.50	117
II	0.51-0.61	135
III	0.62-0.72	168
IV	0.73-0.84	259
V	0.85-1.03	437
Parent (P)	0.41-1.03	156

[1]Conditions of chromatography and fraction characterization.

Column dimensions: 1 X 29 cm (total volume (V_t) = 22.8 cm^3)
Gel: Sephadex G100/150
Eluant: Millipore Super "Q" water, adjusted to pH 8.60 with 1.0 N NaOH.
Flow rate: 0.68 ml cm^{-2} min^{-1}.
Void volume (V_o): Blue dextran 2000; V_e/V_t = 0.43 (V_e = eluted volume).

[2]Described by Stewart and Wetzel (1980a).

Inlet DHM on carbon assimilation and APA were compared to effects of each amendment alone. The volume of humic or phosphate solution added in these experiments was always small (< 0.5 percent, v:v) to preclude dilution artifacts.

Carbon assimilation

Assimilation of ^{14}C was determined by adding 100 l of $NaH^{14}CO_3$ (100 µCi ml^{-1}) and incubating the uncapped vials for 2-4 hr periods. Rapid termination of ^{14}C assimilation was accomplished at the end of the incubation period by addition of 200 µl of concentrated HCl per vial. Contents of all vials were then frozen, lyophilized to dryness, and resuspended in 0.5 ml "Q" water prior to radioassaying for assimilated ^{14}C by adding 10 ml of scintillation media. Assimilated carbon collected on Nuclepore filters was determined by liquid scintillation (10 ml Instagel) after removing residual inorganic label (HCl fuming, 5.0 minutes). In all experiments, appropriate corrections were made to account for background activity and quench (external ratios method) prior to conversion to relative rates of carbon assimilation (dpm ml^{-1} h^{-1}).

Alkaline Phosphatase Activity

APA of 4.0-ml samples was determined fluorometrically by measuring rate of fluorescence increase resulting from enzymatic hydrolysis of the non-fluorescent substrate, 3-O-methyl fluorescein phosphate (Perry 1972). Increase in fluorescence was measured at a setting of 3X with a Turner Model 100 fluorometer with filters (Numbers 47B and 2A-12) selected for excitation and emission wavelengths of 436 and > 510, respectively. Samples were incubated with 0.5 ml of the substrate at

room temperature (21-23°C) for 10-60 minutes, depending upon enzymatic activity. As noted by Pettersson (1980), I found consistent linear relationships between fluorescence intensity and product concentration using these procedures so that conversion from change in fluorescence per unit time to rate of orthophosphate liberated (nM P released l^{-1} min^{-1}) was straightforward. When higher concentrations of humic materials were used, appropriate corrections were employed to compensate for lower quantum yields of the fluorescent hydrolysis product. Day-to-day drift in fluorometer response was corrected by routinely standardizing the fluorometer against a 15 µg l^{-1} solution of quinine sulphate (excitation at 360 nm, emission at 460 nm).

Diel differences

Post-incubation size fractionation (Nuclepore filters 12, 5 and 1 µm) was used to compare diel differences in rates of ^{14}C assimilation in response to a 10.0 mg l^{-1} concentration of Inlet DHM. In this experiment, the contents of replicate vials were filtered onto Nuclepore filters of the appropriate pore-size after incubation with labelled bicarbonate for successive intervals of 2.0 h.

Rates of label dissimilation

Phytoplankton samples were amended with either Inlet DHM or FA (each at 10 mg l^{-1}) in order to assess the effects of these two materials on rate of ^{14}C assimilation and on dissimilation of recently-accrued label. Cyclotella-dominated assemblages amended with either Inlet DHM or FA were incubated at 20°C (100 µE m^{-2} sec^{-1}) for 2.5 days. At the start of the third light phase, $NaH^{14}CO_3$ was added to all samples, and ^{14}C assimilation was allowed to proceed for 14 h. At the end of the light phase, replicate samples of each DHM treatment

were filtered (Nuclepore, 8, 5 and 1 μm pore-sizes) to determine amounts of label accrued in > 8, 5-8 and 1-5 μm particle size-classes. Other replicate samples were incubated in darkness for an additional 10 h prior to filtration, and loss of accrued ^{14}C was used to estimate rates of dissimilation.

RESULTS

DHM Effects on Carbon Assimilation

After three days of incubation under controlled conditions, algal assemblages dominated by Cyclotella sp. exhibited marked diel differences in rates of ^{14}C assimilation (Figure 1). In this assemblage, post-incubation size-fractionation (Nuclepore filters 12, 5 and 1 μm) showed that near the end of the 14-h light phase, the largest size-fraction (> 12 μm) assimilated labelled carbon at a rate more than three times greater than the early light phase rate. Rate of carbon assimilation by particles in the 5-12 μm size-class also increased throughout the light phase, but contributed only 1-13 percent to the total amount of label assimilated by the intact assemblage. The smallest size-fraction (< 5 μm), which did not demonstrate significant diel fluctuations, contributed 30-55 percent to intact assemblage rates of ^{14}C assimilation. In this experiment, Inlet DHM (10 mg l^{-1}) caused a progressive inhibition of ^{14}C assimilation by particles in the > 12 μm size-class, but did not significantly alter rates of label assimilation in the two smaller size classes. The overall depression in rate of label assimilation induced by 10 mg l^{-1} of Inlet DHM amounted to about 12 percent (Figure 1). To prevent diel differences in rates of ^{14}C assimilation from confounding further experiments, additional experiments were routinely conducted over standardized

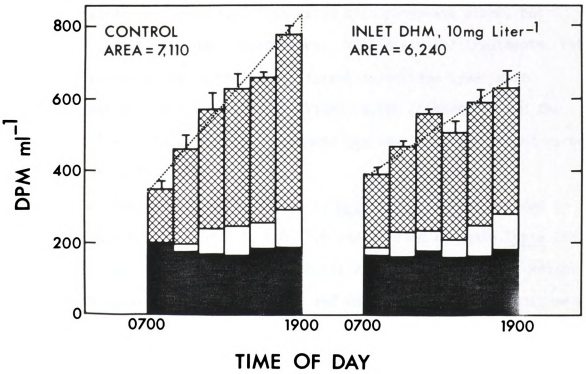


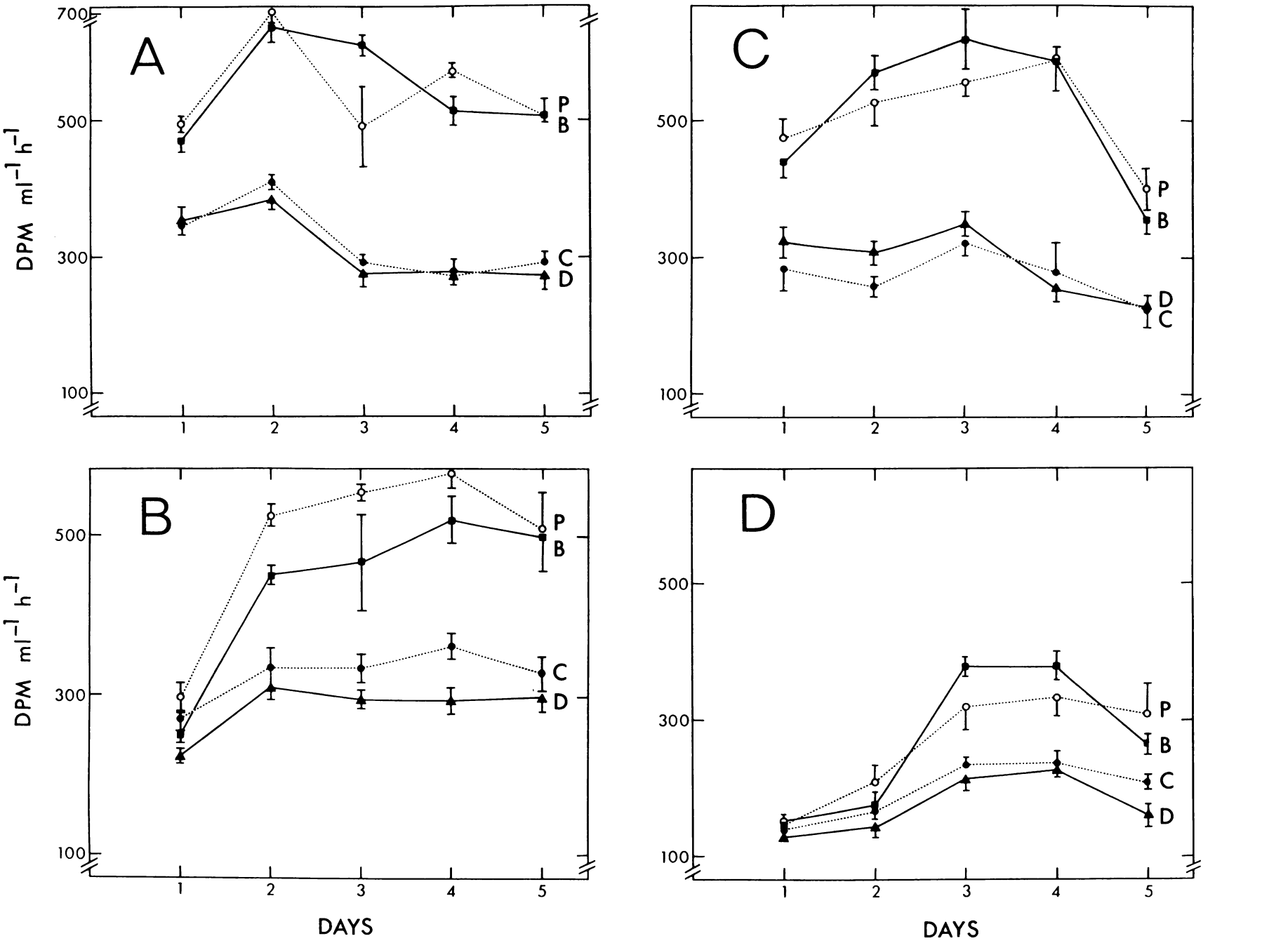
Figure 1. Diel differences in rates of ^{14}C assimilated (DPM ml^{-1} h^{-1}) by mixed algal-bacterial assemblages under high light regimes (100 μE m^{-2} sec^{-1}). Left: unamended samples. Right: samples amended with 10 mg l^{-1} Inlet DHM. Solid areas: particle size-class = 1-5 μm; clear areas: particle size-class 5-12 μm; hatched areas: particles > 12 μm. Error bars designate $\pm$ 1 S.E., n = 6, for total assimilation rates.

intervals of time (1000-1400).

In another series of experiments, two algal-bacterial assemblages (dominated by either Cyclotella sp. (C. michiganiana, C. commensis) or cryptophytes (Cryptomonas sp., Rhodomonas minuta) were amended with 7.2 mg l^{-1} Inlet DHM, 18 µg l^{-1} phosphate, or both DHM and phosphate. Addition of phosphate consistently stimulated rates of ^{14}C assimilation in both assemblage types as compared to unamended controls, while Inlet DHM alone resulted in slight inhibition (Figure 2). Samples amended with both phosphate and Inlet DHM demonstrated rates of ^{14}C assimilation that were less than rates with phosphate alone, but greater than non-amended assemblages. Within similar treatments, rates of ^{14}C assimilation in the two different assemblage types were consistently higher in the higher light regime (Figure 2), and the 18 µg l^{-1} phosphate amendment enhanced the low light-high light carbon assimilation disparity (Figure 3).

When the assemblage dominated by Cyclotella sp. was amended to final concentrations of 2.5, 5.0, 7.5 and 10.0 mg l^{-1} with Typha DHM that had been fractionated on the basis of apparent molecular weight, both DHM apparent molecular weight and DHM concentration effects were apparent (Figure 4). Typha DHM of apparent molecular weight in excess of 100,000 daltons (Fraction I) slightly depressed rates of ^{14}C assimilation, while fractions of lowest apparent molecular weight (Fractions IV and V) markedly increased rates of ^{14}C assimilation relative to samples amended with similar amounts of control Typha DHM. Higher concentrations of Typha DHM fractionated on the basis of apparent molecular weight were consistently more stimulatory to rates of carbon assimilation than were lower concentrations. Maximal

Figure 2. ^{14}C assimilation rates of Cyclotella and crypotphyte assemblages in the presence of phosphate (P), Inlet DHM (D), or both phosphate and Inlet DHM (B). Unamended samples = C. Top left: Cyclotella, light intensity = 100 $\mu E\ m^{-2}\ sec^{-1}$. Bottom left: Cyclotella, light intensity = 25 $\mu E\ m^{-2}\ sec^{-1}$. Top right: cryptophytes, light intensity = 100 $\mu E\ m^{-2}\ sec^{-1}$. Bottom left: cryptophytes, light intensity = 25 $\mu E\ m^{-2}\ sec^{-1}$. Error bars = 1 S.E., n = 3.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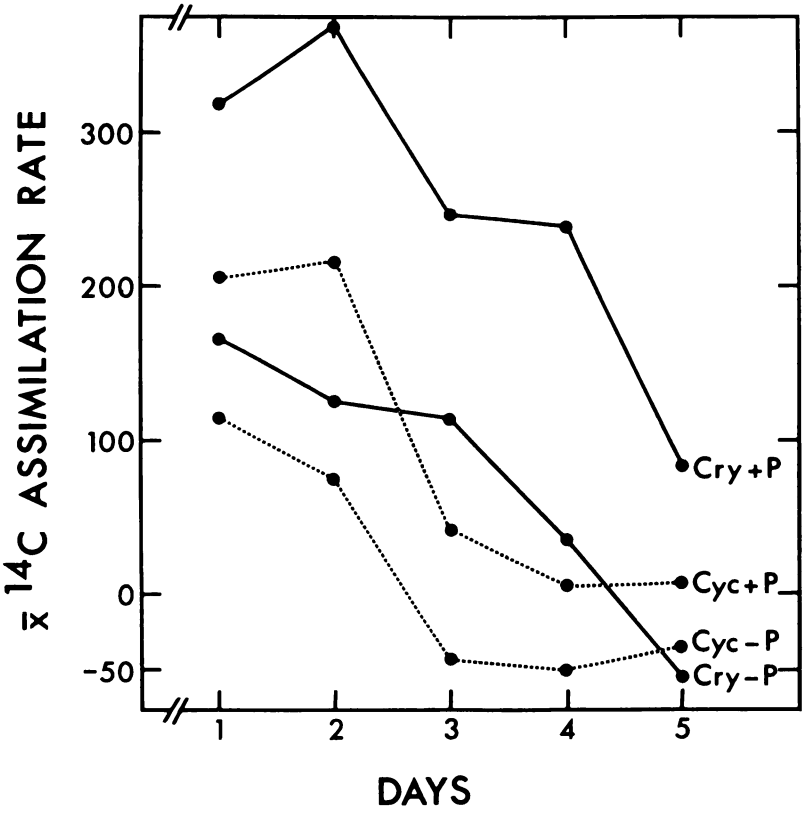


Figure 3. Difference in ^{14}C assimilation rates between high light (100 µE m^{-2} sec^{-1}) and low light (25 µE m^{-2} sec^{-1}) regimes by cryptophyte-dominated assemblages (Cry) and Cyclotella-dominated assemblages (Cyc) with (+P) or without (-P) phosphate amendments. each point represents the mean of 6 replicates, with the assumption of no significant DHM effects.

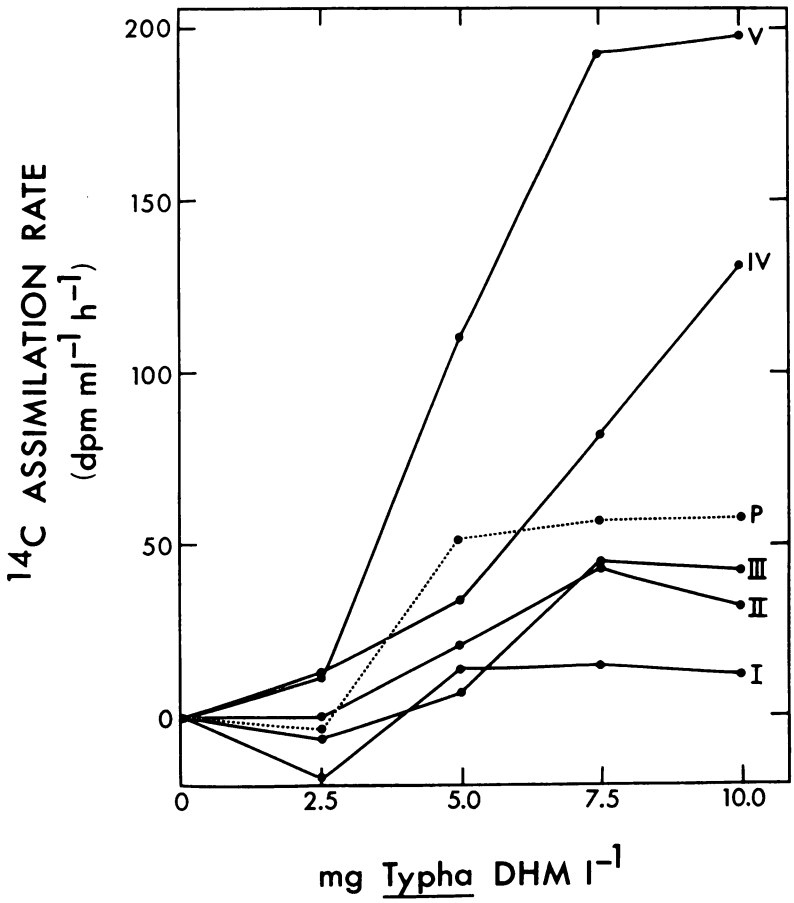


Figure 4. ^{14}C assimilation responses of Cyclotella-dominated algal assemblages to various concentrations of Typha DHM fractionated on the basis of apparent molecular weight. DHM fractions I through V represent DHM of declining apparent molecular weight; P = fractionated, pooled parent Typha DHM. Each point represents the mean of three values, subtracted from a mean of unamended sample responses (n = 9).

enhancement of carbon assimilation in response to increasing concentration of Typha DHM was observed in fractions of lowest apparent molecular weight (Figure 4).

Quantity of label assimilated by Cyclotella-dominated assemblages during a 14-h light phase was reduced to a greater extent by FA than by Inlet DHM (Figure 5), and the extent of inhibition was greater in the smaller particle size-classes (Table 2). Intact assemblages lost about 16 percent of the accrued label after 10 h of darkness. Particles in the > 8 μm size-class lost much less label (about 4 percent) after 10 h of darkness than did particles in the smaller size-fractions, which lost as much as 22-35 percent (Table 3). In general, DHM amendments appeared to impinge to a greater extent upon label assimilation than upon dissimilation of recently-accrued carbon, but some indication of greater rates of dissimilation in the 1-5 μm particle size-classes of DHM-amended samples was observed (Table 3).

DHM Effects on Alkaline Phosphatase Activity

In general, pelagial microflora assemblages rapidly (1-3 days) increased alkaline phosphatase activities (APA) in response to amendments of any of the three unfractionated dissolved humic materials. Increases in APA in unfractionated algal assemblages were concentration-dependent up to levels of 15 mg l^{-1} (Figure 6). Diel differences in APA were not observed, and within-treatment variance of APA was consistently lower than withintreatment variance of ^{14}C assimilation (CV percents of ca. 4.2 and 8.9, respectively).

APA responses of unfractionated algal assemblages were largely independent of algal species composition, but were very sensitive to amendments of either phosphate or DHM. In algal assemblages dominated

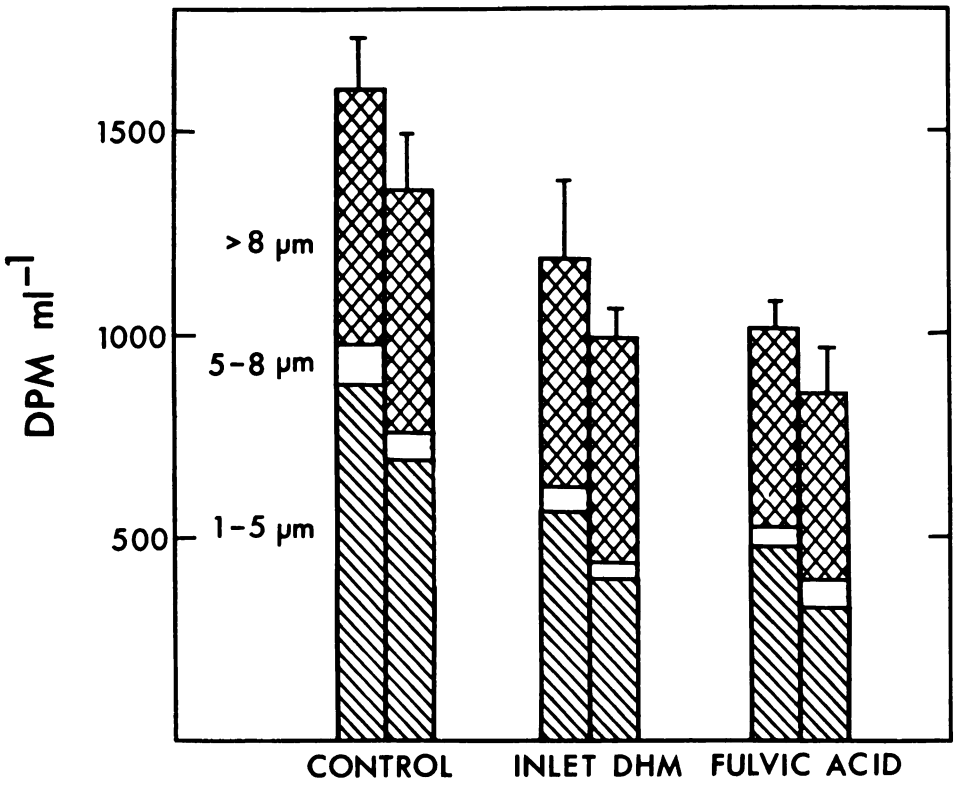


Figure 5. Effect of Inlet DHM and fulvic acid (FA) on ^{14}C assimilation (left bar of each pair) (14 h light) and dissimilation (right bar of each pair) (10 h darkness) in particle size-fractions of > 8 µm, 5-8 µm, and 1-5 µm in Cyclotella-dominated algal assemblages. Error bars represent ± 1 S.E. for intact assemblages.

Table 2. Percent reduction* in rate of ^{14}C assimilation in *Cyclotella*-dominated algal assemblages (14 h light, 100 $\mu E\ m^{-2}\ sec^{-1}$) in the presence of Inlet DHM or FA (each at 10 mg l^{-1}).

Assemblage size-class	Inlet DHM	FA
Unfractionated	25.9	36.6
> 8 μm	9.3	21.0
5-8 μm	42.0	60.0
1-5 μm	35.8	44.9

*Compared to unamended control assemblages.

Table 3. Percent dissimilation of accrued ^{14}C after 10 h of darkness in Cyclotella-dominated algal assemblages in the presence of Inlet DHM or FA (each at 10 mg l^{-1}). (See text for details).

Assemblage size-class	Control	Inlet DHM	FA
Unfractioned	15.3	16.5	16.1
> 8 µm	3.0	1.2	7.7
5-8 µm	35.0	25.8	+175.0*
1-5 µm	21.6	30.1	33.0

*Low absolute assimilation rates in this size class (2.5-6.3 percent of unfractioned assemblage rates) lead to relatively large deviations for all 5-8 µm particle size-class response values.

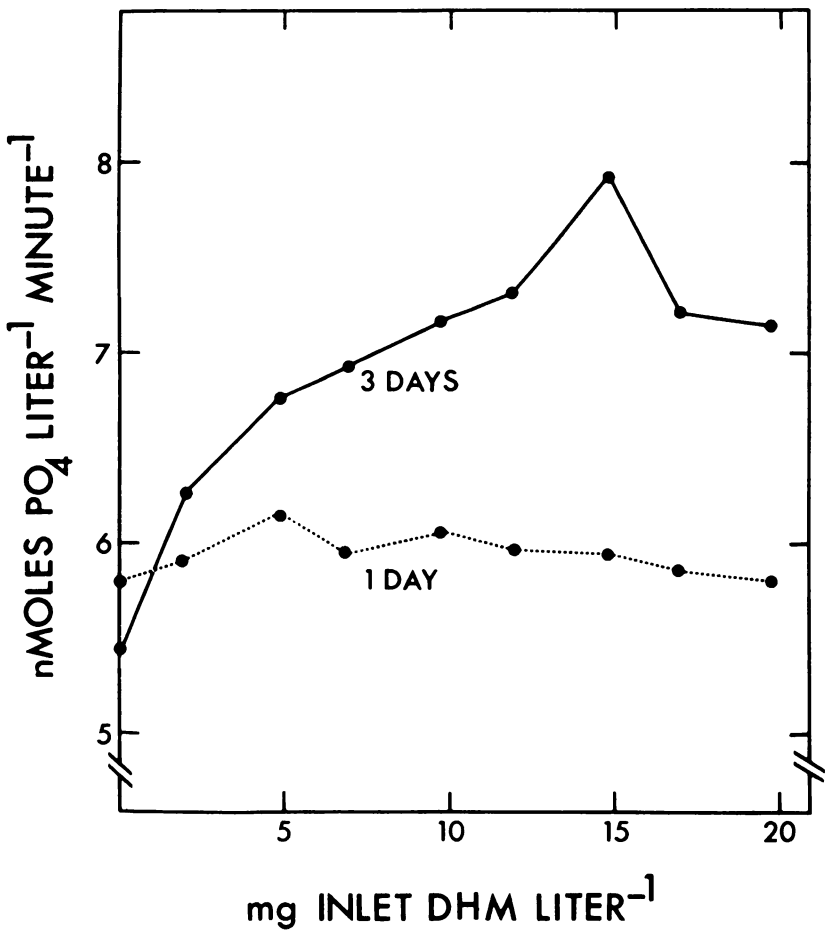


Figure 6. APA responses to varying concentrations of Inlet DHM in unfractionated lagal assemblages dominated by Cyclotella. Day 1 = APA after one day of incubation (20°C, 25 μE m^{-2} sec^{-1}). Day 3 = APA after three days of incubation.

by Cyclotella sp., addition of phosphate depressed APA below APA levels in unamended samples, while addition of unfractionated DHM (7.2 mg l^{-1}) always increased APA relative to unamended samples (Figure 7). Samples amended with both phospahte and Inlet DHM showed increases in APA, but to a smaller extent than with addition of DHM alone.

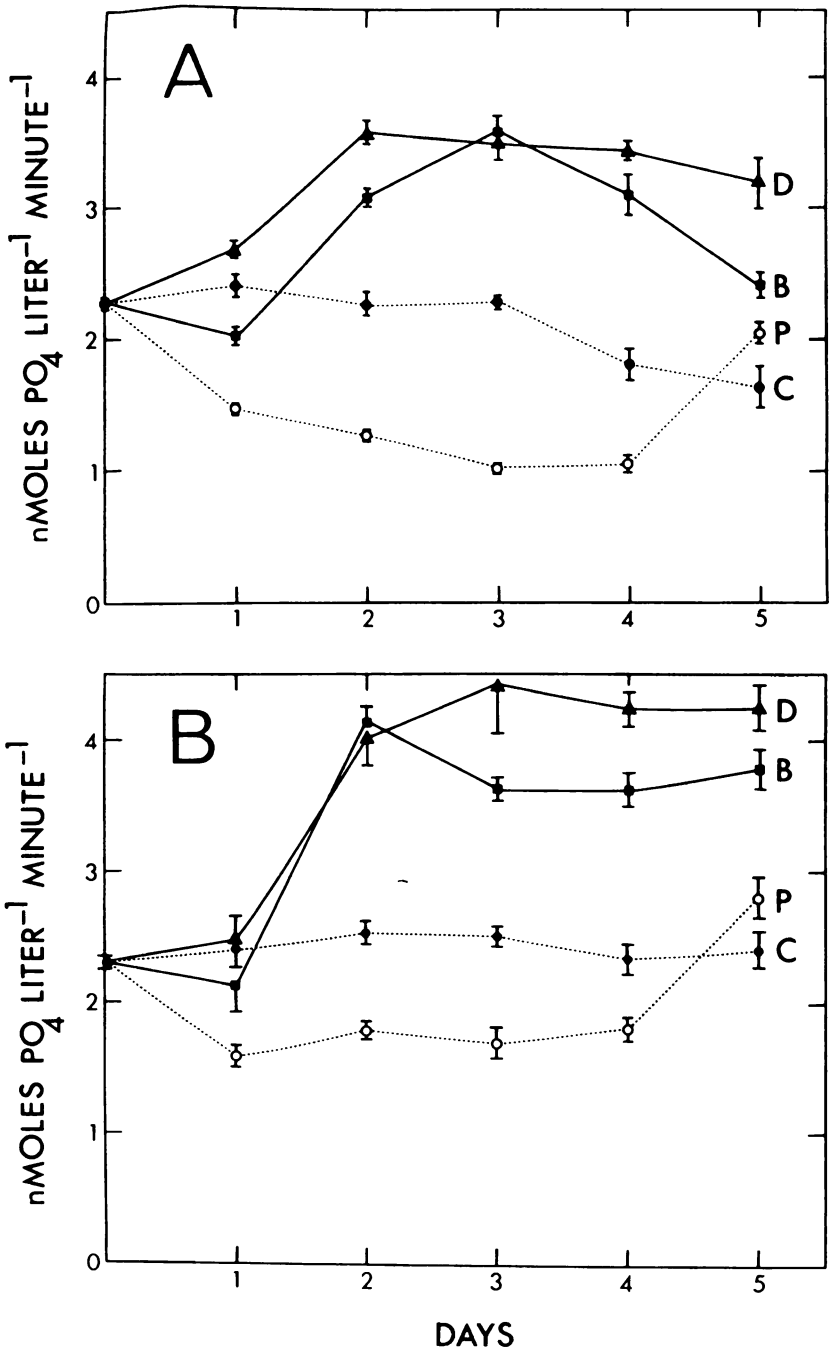
APA responses to amendments of phosphate and/or DHM were markedly influenced by the light regime to which the samples were exposed. Depression of APA by phosphate (18 µg l^{-1} final concentration) was more pronounced in high light regime experiments (100 µE m^{-2} sec^{-1}) than under low light regimes (Figure 8). High light-low light differences in APA response to DHM and phosphate amendments also indicated the presence of relatively large interactive effects, particularly under the high light regime (Table 4).

The Cyclotella-dominated algal-bacterial assemblage responded quickly (2 days) to amendments of MW-fractionated Typha DHM, and magnitude of the APA response was dependent upon both DHM concentration and DHM apparent molecular weight. In any given fraction, higher concentrations tended to be more stimulatory than did lower concentrations, and fractions of lowest apparent molecular weight (fractions IV, V) were much more stimulatory to APA than were fractions of high apparent molecular weight (Figure 9).

DISCUSSION

When using $NaH^{14}CO_3$ techniques to compare rates of carbon assimilation by aquatic microflora exposed to differing treatments, great care must be taken to insure that the treatments that are used do not differentially alter inorganic carbon availability. In the experiments presented in this paper, pH (measured both at the start and

Figure 7. APA responses by Cyclotella and cryptophyte assemblages in the presence of phophate (P), Inlet DHM (D), or both phosphate and Inlet DHM (B). Unamended samples C. Top left: Cyclotella, light intensity = 100 $\mu E\ m^{-2}\ sec^{-1}$. Bottom left: Cyclotella, light intensity = 25 $\mu E\ m^{-2}\ sec^{-1}$. Top right: cryptophytes, light intensity = 100 $\mu E\ m^{-2}\ sec^{-1}$. Bottom right: cryptophytes, light intensity = 25 $\mu E\ m^{-2}\ sec^{-1}$. Error bars = $\pm$ 1 S.E., n = 3.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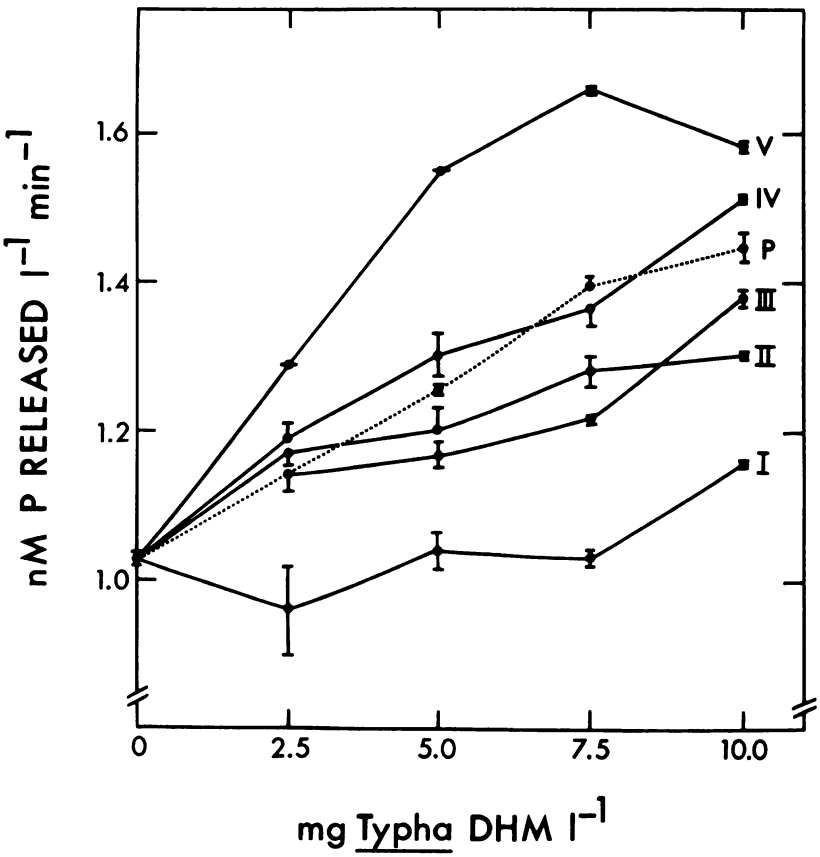

Figure 8. APA responses of Cyclotella-dominated algal assemblages to various concentrations of Typha DHM fractionated on the basis of apparent molecular weight. DHM fractions I through V represent DHM of declining apparent molecular weight; P = fractionated, pooled parent Typha DHM. Each point represents the mean of three replicates, ± 1 S.E.

Table 4. APA Responses[a] (nM P released l^{-1} min^{-1}) to phosphate[b] and inlet and inlet DHM[c] by cryptophyte (cry) and Cyclotella spp. (cyc).

Light regime[d]	factor	Days of Incubations									
		1		2		3		4		5	
		cry	cyc	cry	cyc	cry	cyc	cry	cyc	cry	cyc
low	DHM	0.38	0.11	1.86	1.45	2.25	1.91	1.78	1.90	1.62	1.86
high	DHM	0.20	0.24	1.07	1.27	1.66	1.14	1.29	1.65	1.15	1.22
low	PO_4	-0.23	-0.65	0.56	-0.79	0.05	-0.91	0.01	-0.45	-0.18	0.45
high	PO_4	-0.27	-0.93	0.08	-0.99	0.36	-1.27	-0.17	-0.68	0.13	0.39
low	interactive[e]	-0.08	0.38	0.22	0.87	0.62	0.01	-0.40	-0.22	-0.94	-0.90
high	interactive	0.07	0.27	0.10	0.57	0.57	1.51	0.28	0.34	0.91	-0.86

a. Relative to unamended controls; each value represents a mean of three replicates, CV percent 3.2.
b. Final concentration of 18 μg l^{-1}.
c. Final concentration of 7.2 mg l^{-1}.
d. High = 100 μE m^{-2} sec^{-1} PAR, low = 25 μE m^{-2} sec^{-1} PAR.
e. Interactive term = $|M-C| - \{|D-C| - |P-C|\}$, where M = response to DHM+PO_4, C = response of control samples, D = response to DHM alone, and P = response to PO_4 alone.

at the conclusion of experiments) was not altered by the small amounts of DHM or phosphate that were added. However, DHM does contribute to alkalinity (Wilson, 1979; personal observations), and consequently, some uncertainty originates when attempting to calculate absolute rates of carbon assimilated in these experiments. I have elected to present the data as assimilated ^{14}C rather than as absolute rates for this reason. Since DHM amendments resulted in either stimulation or inhibition of rate of label assimilation (cf. Figures 1 and 4), I believe that DHM did alter rates of carbon assimilation, as well. Secondly, addition of DHM did not exert an immediate effect on rate of ^{14}C assimilation. The delay in response is suggestive more of a biological phenomenon than it is of an interaction between the bicarbonate equilibrium and DHM, although DHM can inhibit the precipitation of $CaCO_3$ in hardwater systems (Reynolds 1978; Stewart and Wetzel 1981).

Maximal enhancement of ^{14}C assimilation in these experiments was found with DHM amendments of lower apparent molecular weight, which agrees with findings of Prakash and Rashid (1968), Prakash (1971) and Prakash et al. (1973), whose works were based on unialgal cultures. It is interesting to note, in addition, that all Sephadex G100 fractions of Typha DHM were more stimulatory to ^{14}C assimilation than was Typha DHM that had not been passed through the Sephadex column. This feature, which was also observed by Droop (1966), indicates that the gel, eluant or DHM processing procedures (e.g. lyophilization, reconstitution) either reduces toxicity or enhances the stimulatory nature of the DHM.

Even in grazer-free systems, increases in rate of carbon

assimilation in response to DHM need not correspond to increases in rate of algal cell numbers or biomass unless respiratory losses (R) and loss rates of excreted organic carbon (EOC) are unaffected by DHM. Our experimental design did not allow the separation of EO^{14}C from particulate organic ^{14}C. In addition, although short-term labelling (14 h) is clearly insufficient to result in a uniformly-labelled assemblage of algae, data presented in the loss-of-label experiments (Table 3) indicates that DHM may alter patterns of dissimilation of recent photosynthate, as well as rates of carbon assimilation.

It is clear that DHM is general, and low molecular weight DHM in particular, are important complexation and chelation agents for trace metals (Rashid 1971; Steinberg and Stabel 1978; Mantoura and Riley 1975). There is, consequently, always a strong temptation to ascribe effects of DHM on algal cultures as a phenomenon related to trace metal availability. This argument may indeed be valid (but see Paasche 1977), and a chelation-based hypothesis can undoubtely be generated to explain some of the observations in this study. However, the enhancement of APA by all additions of DHM (MW-fractionated or not, high concentrations or low, Inlet DHM, Typha DHM, and fulvic acid) are suggestive that DHM effects on rates of ^{14}C assimilation are mediated more by phosphorus avilability than by trace metal availability in the Lawrence Lake ecosystem. Since increases in APA generally correspond to decreasing avilability of phosphorus (cf. Berman 1970; Heath and Cooke 1975; Pettersson and Janson 1978; Pettersson 1980; Wetzel 1981), DHM-phosphorus interactions appeared likely.

Inlet DHM and Typha DHM at concentrations of 10 mg l^{-1} contained undetectable quantities of ammonia or orthophosphate. In addition,

when 10 mg l^{-1} concentrations of these two materials were incubated (60 minutes, 30°C, pH 8.6) with an excess of calf thymus alkaline phosphatase (E.C. 3.1.3.1; Sigma), no detectable quantities of phosphate were released. Since the phosphate assay used had detection levels of ca. 1 µg PO_4 l^{-1} (Wetzel and Likens 1979), I conclude that concentrations of enzyme-assessable phosphomonoesters associated with the two DHM sources was negligible. In addition, if phosphate was released from the DHM by APA in the microflora assemblages, amendments of DHM should have consistently stimulated ^{14}C assimilation, for the assemblages always increased rates of ^{14}C assimilation in response to amendments of phosphate. Concomitant increases in rates of ^{14}C assimilation and in APA were observed only in lower molecular weight fractions of Typha DHM, which does not support the hypothesis of organic phosphorus contamination.

It is simplistic to consider APA a strict measure of community phosphate stress, for enzymatic activity is dependent upon water temperature and pH, ionic composition (Gravalas and Manetas, 1980), and in some cases can be induced by starvation for pyrimidines or guanine as well as by phosphate (Wilkins 1972). In addition, bacterial APA is occasionally constitutive (Pratt 1980), and other evidence indicates potential regulation of APA by the metabolite cAMP (Francko and Wetzel, unpublished data). All these factors nothwithstanding, high levels of APA in lakes are likely associated with low concentrations of phosphate (cf. Karl and Craven 1980).

Since concentrations of DHM used in these experiments (2-15 mg l^{-1}) were 2-3 orders of magnitude greater than concentrations of total phosphate (ca. 10-35 g l^{-1}), even low binding affinities between DHM

and orthophosphate could place substantial constraints upon available phosphate. In other experiments, a form of a zonal analysis was used to estimate binding between the DHM and carrier-free ^{32}P-orthophosphate (Stewart and Wetzel 1981), and results clearly indicated that DHM-orthophosphate interactions were unimportant in the Lawrence Lake system.

Increases in community APA in response to increases in DHM may be due to either abiotic or biotic mechanisms. In the former category, for example, DHM may sequester organic phosphorus-containing molecules and render phosphorus available only through enzymatic hydrolysis. In this case, production and release of organophosphorus materials by the microflora would gradually result in decreased phosphate availability, for the DHM pool would initially serve as a net sink for phosphorus. Equilibrium would then be established only after increases in APA allowed more phosphorus to become available to the microflora.

In the second category, one possibility is that the presence of DHM stimulated the growth of either bacteria or algae, and that increases in competition between members of these two groups for a limiting resource (e.g. phosphate) caused either group to increase its APA. Since ^{14}C assimilation was not enhanced in the presence of unfractionated Inlet DHM (although APA did cause an increase), the photoautotrophic members of the assemblage appear to be unlikely candidates for growth stimulation. Increases in bacterial growth in the presence of the DHM may have occurred (cf. Figure 5, Table 2), but insufficient data are available to make a more definitive statement.

When DHM effects on rates of ^{14}C assimilation were ignored, phosphate amendments were consistently more stimulatory under the high

light regime than under the low light regime in both assemblage types (Figure 3). Rhee (1978) did not find additive effects when axenic cultures of Scenedesmus were simultaneously limited by phosphorus and nitrogen. However, in other algal species energy allocation patterns are dependent upon available energy (cf. Ward 1978; Ward and Wetzel 1981). Although simultaneous limitation by two factors (light, phosphorus) is suggested by the data in Figure 3, the nature of the assemblages used (mixed bacterial and algal), the relatively short duration of the experiments (5 days), and the inevitable ambiguities of phosphate limitation (luxury consumption, the presence of various quantities of organo-phosphorus compounds, and differing alkaline phosphatase activities) render such a conclusion premature. In addition, the consistent depression of the high light-low light disparity with time (Figure 3) could be interpreted as a progressive exhaustion of yet another resource, such that (were one to extrapolate beyond 5 days), rates of ^{14}C assimilation by either assemblage type would show no significant differences between light regimes or phosphate treatments after six or seven days. Other experiments are required to conclusively test any of these possibilities.

In the two assemblage types, APA responses to amendments of DHM or PO_4 were very dependent upon the light regime to which the assemblages were exposed. Phosphate additions depressed APA in Cyclotella-dominated assemblages grown under and high light regimes (mean values of 0.47 and 0.90 nM P released l^{-1} min^{-1}, respectively), but did not significantly depress APA in cryptophyte assemblages in either light regime (Table 4). In both assemblage types, amendments of DHM induced higher APA in low light regimes than under high light

regimes (mean values of ca. 1.52 vs 1.08 nM P released l^{-1} min^{-1}, respectively), and when considered over the entire five days, interactive terms were significant and positive (ca. 0.38 nM P released l^{-1} min^{-1}) only under the high light conditions (Table 4). DHM of low apparent molecular weight was more stimulatory to APA than DHM of high apparent molecular weight (Figure 8), and DHM of low apparent molecular weight is rapidly degraded by sunlight (Stewart and Wetzel 1981). It is possible, therefore, that under the higher light regime, APA stimulation by DHM was reduced due to selective photodegradation of low molecular weight, simulatory DHM constituents. The DHM-PO_4 interactive terms in both assemblage types were largely positive over days 1-3, and tended to become strongly negative by day 5 (Table 4). These data clearly demonstrate that the non-additive APA responses to DHM and PO_4 cannot safely be extracted from a temporal matrix and used as constants; APA responses by the assemblages were both dynamic and complex.

In either case, the ecological ramifications to both system productivity and phosphorus cycling appear to be important, for major rain events can simultaneously introduce substantial quantities of phosphorus and DHM into pelagial areas of small and moderate-sized lakes (Stewart and Wetzel 1981). Interactions between DHM and phosphorus cycling in aquatic systems may be as important as trace metal-DHM interactions, and clearly warrant a much closer scrutiny.

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CHAPTER VI

Phytoplankton contribution to alkaline phosphatase activity

INTRODUCTION

Numerous studies suggest that algal production of phosphomonoesterases (E. C. 3.1.3.1.) increases as phosphorus deficiency occurs. Presumably, increases in activity of alkaline phosphatase under conditions of low phosphate concentrations (either from increased rates of synthesis of the enzyme or from derepression of pre-existing enzyme) enhance algal competitive ability by releasing phosphate from organophosphorus compounds (cf. Berman 1970; Jones 1972; Heathe and Cooke 1975; Jansson 1976; Pettersson and Jansson 1978; Pettersson 1980). However, although free dissolved alkaline phosphatase is short-lived (Reichard et al. 1967; Pettersson 1980), substantial quantities (sometimes in excess of 50 percent of the total activity) can be found in the dissolved phase (Pettersson 1980; Wetzel 1981; this study). In addition, Jones (1972) has shown that bacterial production of alkaline phosphatase in pelagial environments does occur, and bacterial production of alkaline phosphase production need not occur in response to low levels of phosphorus (Kuo and Blumenthal 1961; Wilkins 1972; Forsberg and Cheng 1980).

Algal phosphatase activity is usually assessed by filter-fractionating whole water samples into dissolved and particulate

components, and assuming that bacterial contribution to the particulate phase is negligible (cf. Heathe and Cooke 1975; Pettersson 1980). Consequently, the extent to which the presence of non-algal particulate alkaline phosphatase activity biases estimates of algal alkaline phosphatase activity has to date remained untested. In this paper I present evidence demonstrating that in many cases algal contribution to the pool of particulate alkaline phosphatase activity is in fact relatively small.

MATERIALS AND METHODS

Water samples were collected at two or more depths from several lakes in Kalamazoo and Barry counties, southwestern Michigan (Table 1). Prior to analyses, all water samples were filtered through 160 μm mesh-size Nitex to remove large grazers. Nitex-filtered samples were gently stirred using a magnetic stirrer to insure homogeneity of subsamples. All analyses were conducted within 1 h after sample collection.

Alkaline phosphatase activity (APA) of 4.0 ml samples was determined fluorometrically by measuring rate of fluorescence increase resulting from enzymatic hydrolysis of the non-fluorescent substrate, 3-O-methyl fluorescein phosphate (Perry 1972). Increase in fluorescence was measured at a setting of 3X with a Turner Model 110 fluorometer with filters (Numbers 47B and 2A-12) selected for excitation and emission wavelengths of 436 and >510 nm, respectively. Samples were incubated with 0.5 ml of the substrate at room temperature (21-23 C) for 10-60 minutes, depending upon enzymatic activity. As noted by Pettersson (1980), I found consistent linear relationships between fluorescence intensity and product concentration using these procedures, so that

conversion from change in fluorescence per unit time to rate of orthophosphate liberated (nM P released liter $^{-1}$ minute^{-1}) was straightforward.

Photosynthetic activity of the samples was assessed by incubating 40 ml of sample in an environmental chamber at 20 C for 2.0-2.6 h in the presence of 100-200 µl of $NaH^{14}CO_3$ solution (100 µCi ml^{-1}). At the conclusion of the incubation period, triplicate 12-ml subsamples of the labelled cell suspension were rapidly dispensed into graduated 15 ml centrifuge tubes. The 12-ml samples were centrifuged for 3.0 minutes at 2,200 x g. Earlier experiments demonstrated that samples centrifuged in this manner lose only 4-6 percent of their photosynthetic rates compared to non-centrifuged samples, indicating minimal cell damage (cf. Jüttner and Friz 1974). Immediately after centrifugation, three successive, equal volumes (representing top, middle and bottom 4.0-ml strata) were carefully withdrawn from each centrifugation tube using an adjustable 5 ml pipet. Replicate top, middle and bottom strata volumes were dispensed into individual scintillation vials, each of which contained 200 µl of concentrated HCl. The contents of all vials were frozen and lyophilized to dryness to remove both residual inorganic ^{14}C and HCl. Quantity of assimilated carbon was determined by adding 0.5 ml of Millipore "Q" water and 10 ml of scintillation media (Aquasol) to each vial, and radioassying the contents (Beckman LS8000). Appropriate blanks and standards were used to convert values to relative rates of carbon assimilated (dpm ml^{-1}h^{-1}).

Identical centrifugation procedures were used to assess partitioning of alkaline phosphatase activity, with replicate 4.0-ml

centrifuge tube strata being fluorometrically assayed for APA as described earlier. In some experiments 8.0 ml samples were centrifuged at 20,000 x g (5 minutes), and supernatant APA values were compared to APA values obtained when samples were filtered through either pre-combusted (525 C) Reeve Angel 0.5 μm pore-size glass fiber filters (984-H) or 0.22 μm pore-size Millipore GS filters.

For convenience, extent of centrifugation partitioning of each of the two parameters (APA assimilated carbon) was calculated on a 0-10 scale using the equation

$$\text{partition coefficient} = 10 - \left[\frac{T + M}{2B} \times 10\right]$$

where T, M, and B represent values of either dpm $ml^{-1}h^{-1}$ or nM P released l^{-1} min^{-1} for top, middle and bottom strata of the centrifuged samples, respectively. Using both APA and ^{14}C-assimilation data, maximum possible algal contribution to the total APA was calculated for each sample using the equation

$$\text{algal contribution percent} = \left[\frac{B_a - \left(\frac{T_a+M_a}{2}\right)}{B_c}\right] \times \left[\frac{100}{T_a+M_a+B_a}\right]$$

where T_a, M_a and B_a represent APA values (nM P released l^{-1} min^{-1}) for top, middle and bottom strata of each centrifuged sample, and B_c represents the fractional value of carbon assimilated by stratum B (i.e., B_c = dpm ml^{-1} h^{-1} in B divided by the sum of dpm ml^{-1} h^{-1} in strata T, M and B).

RESULTS AND DISCUSSION

Representative patterns of APA and assimilated carbon partitioning are shown in Table 1 and Figure 1. In all cases, assimilated carbon partitioned much more completely (mean partition coefficient = 8.81 $\pm$ 0.10 (SE), n=10) than APA (mean partition coefficient = 2.53 $\pm$ 0.49 (SE), n=10). The data suggest the presence of substantial quantities of dissolved or small particulate APA segregating independently of dominant photosynthetic organisms. Algal contribution to total APA in freshly-collected samples consistently fell below 32 percent (Tables 1 and 2), with some samples as low as 3 or 4 percent.

It is important to note that algal contribution percentages represent maximum possible values, for three assumptions are incorporated into the algal contribution percent formula:

1. The assumption of zero partitioning by bacterial, detrital and dissolved APA.
2. The assumption that viable algae are appropriately represented by the partitioning patterns of assimilated carbon.
3. The assumption of a constant ratio of APA per unit ^{14}C assimilated for all algal species.

The first assumption is undoubtedly violated to some extent, for even gentle centrifugation will sediment out larger detrital particles and associated bacteria, some of which must contribute to total APA Harvey and Young (1980), for example, have shown that in subsurface water collected from a salt marsh tidal creek, a majority of the respiring bacterial cells were particle-bound. The proportions of

Table 1. Representative partition coefficient values for assimilated carbon and alkaline phosphatase activity (APA) of water samples collected from different lakes in Barry and Kalamazoo counties, Michigan.

Sample		Partition Coefficient Value	
		Assimilated Carbon	APA
Lawrence Lake[a]	2m	8.87	0.95
	4m	8.72	1.01
	6m	9.44	2.42
Lefebre Lake[b]	2m	8.94	1.68
	4m	8.87	0.83
Little Mill[a]	2m	8.32	3.06
	4m	8.39	1.89
Gull Lake[a]	4m	8.98	4.27
	9m	8.55	4.03
	15m	8.97	5.20
Mean ± S.E.		8.81 ± 0.10	2.53 ± 0.49

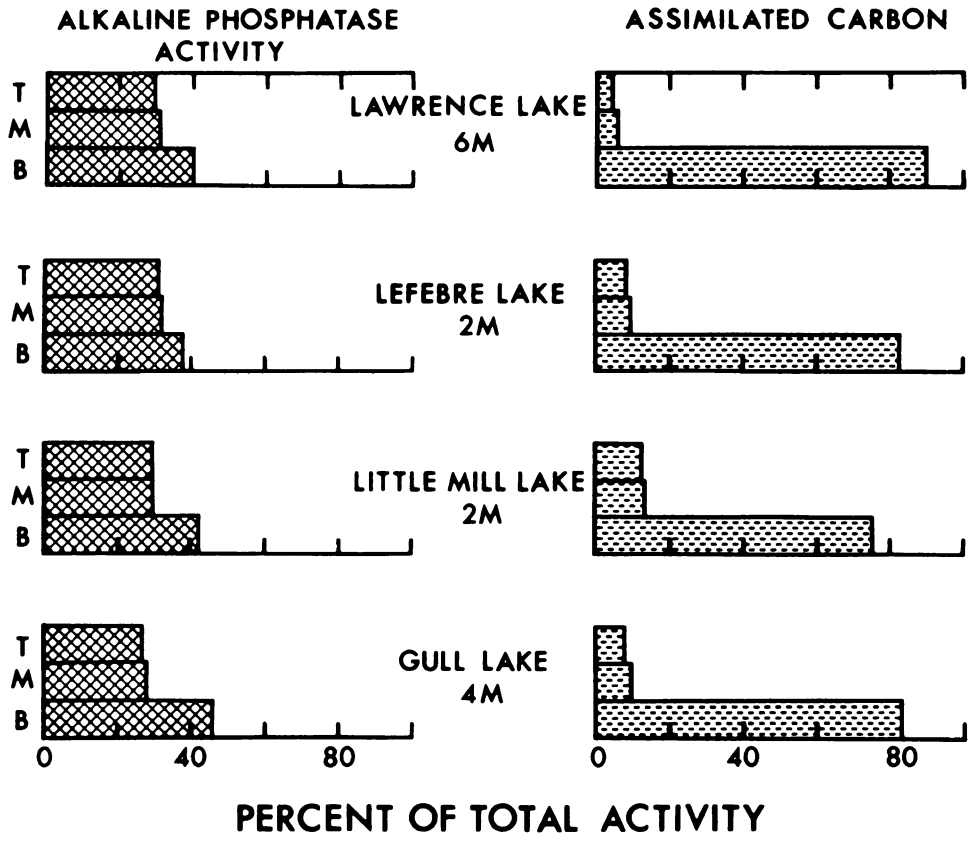

Figure 1. Distribution of alkaline phosphatase activity and assimilated carbon under centrifugation (2,200 x g, 3 minutes). T, M, and B (ordinate) represent top, middle and bottom strata of 12 ml centrifuged samples. Abscissa values represent percent of total activity of each of the two parameters.

Table 2. Percentage of total alkaline phosphatase activity contributed by algae, non-algal particulate, and "dissolved" enzyme in four lakes in Barry and Kalamazoo counties, Michigan. "Dissolved" values are calculated from APA in the supernatant portions of centrifuged samples (20,000 x g, 5 minutes).

Sample		maximum algal	non-algal particulate	dissolved
Lawrence Lake	2m	4.1	44.1	51.8
	4m	4.5	46.3	49.2
	6m	10.7	41.2	48.1
Lefebre Lake	2m	7.6	48.1	44.3
	4m	3.6	54.8	41.6
Little Mill	2m	17.1	68.5	14.4
	4m	9.5	76.8	13.7
Gull Lake	4m	23.9	36.5	39.6
	9m	23.7	24.3	52.0
	15m*	32.0	6.8	61.2

* Low total APA values for this sample (0.16 nM P released l^{-1} min^{-1}); all other samples had APA values $\geq$ 0.83 nM P released l^{-1} min^{-1} prior to centrifugation.

particle-bound and unattached bacteria were not assessed in this study, so that the magnitude of non-algal particulate APA can be calculated only by difference. Filtration procedures are certain to augment the problem of determining relative contribution to total APA by bacteria and algae, for with filtration all particulate matter of dimension greater than the filter pore size is assumed to be algal.

The second assumption is likely violated to a smaller extent, for short-term incubation of the water samples with ^{14}C should not allow much label incorporation into bacterial particles via algal excretion of labelled organic matter. In addition, the nearly quantitative sedimentation of assimilated carbon in most samples (Table 1, Figure 1) indicates that the second assumption is reasonable.

Although a number of algal species have been shown to produce alkaline phosphatase in response to phosphorus limitation under laboratory conditions, it is possible that not all algal species have that capability (cf. Ihlenfeldt and Gibson 1975). In addition, insufficient data are available to suggest a relationship either between APA and cell size or between APA and specific algal groups (e.g. diatoms, chrysophytes, greens, etc.). Consequently, the third assumption (an assumed constant ratio of APA per unit carbon assimilated) could be in error. However, smaller algal cells, which would partition less completely than larger cells in terms of assimilated carbon, would have to account for much more APA than the larger cells to significantly alter the maximum algal percent contribution value, simply because the disparity between partition coefficient values for APA and assimilated carbon is relatively large

(Table 1).

Preliminary data by the author indicate that the extent of algal partitioning may be calculated using measurements of DCMU-enhanced fluorescence of *in vivo* chlorophyll. In this case, the 4.0-ml centrifuged strata are immediately amended with DCMU to a final concentration of 10^{-5} M, and chlorophyll fluorescence is measured after 5 minutes with a Turner Model 111 fluorometer equipped with a red-sensitive photomultiplier and a high sensitivity door. With this procedure, the use of isotopes can be avoided and time requirements are substantially reduced. However, nonuniform chlorophyll fluorescence yields do occur (cf. Prezelin and Ley 1980), and may complicate interpretation to some extent.

Measurements of dissolved APA were performed on filtered (0.22 µm Millipore GS or 0.5 µm pore-size glass fiber Reeve Angel 984-H) and centrifuged (20,000 x g, 5 minutes) samples to determine correspondence of the two procedures. Lawrence Lake centrifuged supernant APA values averaged slightly less ("dissolved" = 54.9 percent of total APA) than did samples filtered through the Reeve Angel filters ("dissolved" = 60.4 percent of total APA), but filtration through the GS filters produced significantly smaller estimates. Boiled lakewater samples and Millipore Super "Q" water samples amended with known amounts of dissolved APA (Sigma) similarly lost nearly 100 percent of the initial APA when filtered through the GS filters. Acid rinsing the filters prior to sample filtration (10 ml 1.0 N HCl) did not improve dissolved APA recovery, and since most of the APA could be recovered by assaying the filter for APA, I conclude that the free enzyme adsorbs to the GS

filter.

In oligotrophic systems free-living bacteria may be extremely small, and several investigators (cf. Ferguson and Rublee 1976; Azam and Hodson 1977) have shown that a majority of the cells have dimensions of less than 0.5 µm. Consequently, measurements of the dissolved APA pool by filtration through Reeve Angel filters (pore size ca. 0.5 µm; Sheldon 1972) are probably overestimates. In Table 2, the amount of dissolved APA in each sample was determined using centrifugation (20,000 x g, 5 minutes), for under these conditions supernatant APA values were consistently lower than Reeve Angel filtrate values by 5-9 percent.

In conclusion, it appears that non-algal particulate APA can be a major component of the particulate pool, for the technique that is commonly used to assess algal contribution to particulate APA overestimates the amount of APA that is directly affiliated with algal cells. It seems likely, in addition, that "dissolved" APA values are also overestimates due to the small size of many free-living aquatic bacteria. The ecological significance of alkaline phosphatase activity in aquatic systems probably cannot be fully appraised until the relative contribution by autotrophs and heterotrophs can be more accurately determined.

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CHAPTER VII

CONCLUSIONS

The relative molecular weight of dissolved humic materials (DHM) in water samples can be conveniently determined using measures of sample absorbance and fluorescence under standardized conditions of pH. This finding is an important step in the elucidation of the role of DHM in aquatic systems, for there are indications of a correspondence between DHM molecular weight and DHM "quality". Low molecular weight DHM, for example, is both more labile and better at trace metal complexation than is DHM of high molecular weight. Data in this dissertation (Chapter V) also indicates that DHM of low apparent molecular weight is much more stimulatory to rates of carbon assimilation and alkaline phosphatase activity (APA) in natural assemblages of algae and bacteria than is DHM of high molecular weight. Consequently, factors that control the molecular weight spectrum and concentrations of DHM are likely to be intimately related to pelagial responses to inputs of DHM.

It is clear that DHM photodegration and interactions between DHM and sediment/pH are important in determining the relative molecular weight of DHM. Other considerations not covered in this dissertation, however (e.g. duration between rain events, nature of the drainage basin and littoral vegetation, seasonal changes in sediment pH, epilimnetic mixing patterns), are also likely important in altering DHM quality and quantity. There is, in addition, circumstantial evidence suggesting that DHM-$CaCO_3$ interactions could be important in altering both DHM

molecular weight and concentration.

Finally, evidence presented in Chapter V indicates that inputs of DHM may influence phosphorus cycling in hardwater lakes. If so, there are important and immediate ecological ramifications of DHM in aquatic systems, for pelagial productivity is often limited by low levels of available phosphorus. Two possibilities for furture investigation seem particularly relevant in order to explore the ecological aspects of DHM-phosphorus interactions: (1) direct interactions between DHM and organophosphorus materials, and (2) the potential competitive interactions between primary producers (algae) and decomposers (bacteria) for a limiting resource (phosphorus), whose relative availability may be altered by inputs of DHM.

Magnitude of responses of algal-bacterial assemblages to DHM were also dependent upon DHM concentration. Unfortunately, concentrations of DHM can not yet be determined using measures of sample absorbance or fluorescence, due to a dependence of DHM spectral characteristics on DHM apparent molecular weight. In the future, if similarly rapid and inexpensive methodologies can be developed to accurately determine DHM concentrations, a powerful avenue towards full elucidation of the role of DHM in aquatic systems can emerge.