TRIHALOMETHANE-PRECURSOR PRODUCTION BY *HYDRILLA VERTICILLATA* IN THE OCCOQUAN RESERVOIR

by

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(ABSTRACT)

This project reviewed the ability of hydrilla to release THM-precursors should this plant become established in the Occoquan Reservoir. It was found that the Occoquan Reservoir could support hydrilla. Three sources of THM-precursors were found to result from hydrilla. These included: 1) extracellular products (ECP) from hydrilla itself; 2) ECP from periphyton that colonized the hydrilla; and 3) decay products from hydrilla. This study projected a potential increase in THMFP of 23 ug/L should hydrilla become established in the Occoquan. The current level of THMFP in the reservoir is approximately 1,000 ug/L.

In addition, the organic compounds released by hydrilla (both ECP and decay products) were characterized on the basis of size. The hydrilla ECP were found to be quite small in comparison to most other organic compounds which occur in the aquatic environment. Those resulting from the decay of

hydrilla were found to vary widely in size, but, in general, approximated the size of organic compounds which occur in the aquatic environment.

ACKNOWLEDGEMENTS

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INTRODUCTION

The Occoquan Reservoir

The Occoquan Reservoir was formed in 1950 by damming the Occoquan Creek to provide a source of drinking water to Northern Virginia. In 1957, the dam height was increased thus expanding the capacity of the reservoir to accomodate the growing demand for water. The reservoir is a multi-purpose impoundment in that it 1) serves as a source of drinking water for over 600,000 people; and 2) is used for recreation. In addition, the Occoquan Reservoir receives treated wastewater from an advanced wastewater treatment plant.

Water Quality Problems

The reservoir has experienced several water-quality problems associated with hypereutrophication. Specifically, high total These include high mineral content, algal blooms, and high dissolved organic carbon (DOC) levels. The high DOC levels in the reservoir are the cause of extremely high trihalomethane formation potentials (THMFP) (about 1,000 ug/L under worst case conditions) before treatment. The high THMFP of the water in the reservoir have presented a problem

to the New Lorton and Occoquan Water Treatment Plants which use the treatment. To avoid exceeding the maximum contaminant level (MCL) for trihalomethanes (THM), which is currently 0.10 mg/L, chloramines must be used for system residuals to avoid the formation of excess THMs. Therefore, additional contributions to the THM precursor pool would add to an already serious problem.

Hydrilla

In the early 1960's, Hydrilla verticillata (hydrilla) was introduced in Florida. By 1980 it had infested over 3 X 10^9 m^2 of that state's lakes and rivers. Hydrilla, which has become the most troublesome aquatic vascular plant in Florida, creates problems found with the over-abundance of any aquatic vegetation. These include the blockage of water flow for flood control and the decreased use of waterways for recreation and commercial purposes.

It was found in the late 1970's that algae could significantly increase the THMFP of the water it inhabited, spawning concern over biologically induced THM-precursors. The appearance of hydrilla in the Potomac and its possible proliferation in the Occoquan Reservoir was cause for such concern, especially to the Fairax County Water Authority, which relies both on the Potomac River and on the Occoquan Reservoir as sources of raw water. Therefore, the Authority provided a research grant to Virginia Tech to explore the

potential seriousness of hydrilla-induced THM problems in the Reservoir itself.

The objectives of this research project were: 1) to determine in the laboratory if the sediment and waters of the Occoquan could support hydrilla growth, 2) to quantify the levels of THM precursors that could be expected from the release of organic substances from both ECP production and decay, and 3) to characterize these organic substances on the basis of their molecular-size distribution and THM yield.

CHAPTER II

LITERATURE REVIEW

Trihalomethanes

In the mid-1970's, the chlorination of public water supplies came under careful scrutiny when trihalomethanes, (a form of chlorinated organic that has been associated with cancer in laboratory rats) were discovered in chlorinated drinking water (1,2). This discovery led to a 1975 Environmental Protection Agency (EPA) survey of the nation's drinking waters. This survey established both the ubiquity of halogenated organics in chlorinated drinking water and the correlation between haloform concentration and the organic content in the raw waters (3). Although several halogenated organics are produced by the chlorination of some waters, the EPA chose to regulate THMs for three reasons. First, they were associated with cancer in laboratory rats; second, they were believed to be good predictors of haloform occurrence; and third, they were more easily detected and measured at that time than any other major organic halogen. Since the establishment of this 0.10 mg/L MCL, many operators, particularly those treating waters containing high concentrations of organic substances, have had trouble meeting the MCL without altering their treatment process.

THM Precursors

Humic Compounds

Since 1975, many investigators have identified and characterized many of the organic substances involved in the THM reaction. By 1976, Johannes Rook had established a link between the presence of humic acids and trihalomethane formation potential of the chlorinated water (4,5). Humic compounds can enter a natural waterbody through several different routes. These include a) direct precipitation, b) incorporation into rain-drops, which then precipitate on the lake, c) in surface or subsurface waters, and d) through the metabolic activity of aquatic organisms. Once in a lake, these organics are either retained in the water column, precipitated into the sediment, or flushed out the outlet.

Figure 1 shows that humic compounds comprise about 50 percent of the dissolved organic carbon (DOC) in lakes and rivers. The remaining 50 percent of the organic substances is composed of hydrophilic acids (30 percent) and simple compounds such as carbohydrates and amino acids (20 percent) (6). The contribution of aquatic macrophytes such as Hydrilla verticulatta to this pool has been ignored, mainly due to their relatively small contribution to the total biomass in water supplies. Hydrilla, however, proliferates to such an extent that it may become a significant source of DOC.

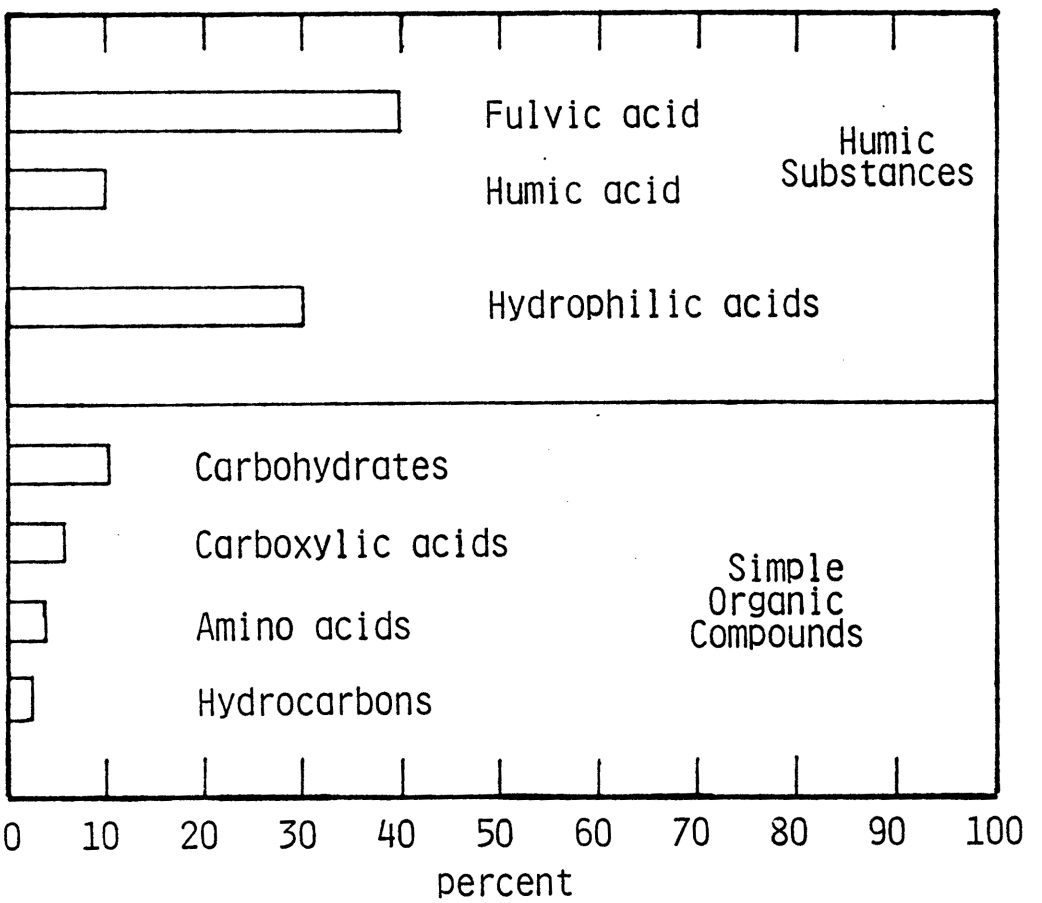


Figure 1. Dissolved organic carbon histogram for average river water with a DOC of 5 mg/L (6).

Humic substances include a wide variety of organic compounds that can be characterized on the basis of many criteria. Originally, because these compounds were initially of interest to aquatic biologists, humic substances were catagorized on the basis of whether they were labile or refractory. Labile organic substances included those which served as substrates for bacteria and, consequently, had a high turnover rate in the environment. On the other hand, those organic substances classified as refactory were not readily biodegradable and, therefore, had low turnover times. As analytical techniques became more sophisticated, constituents of organic pools were catagorized by their solubilitys at different pH values. The fraction that was soluble only under basic conditions was termed the humic acid portion and the fraction that was soluble under both acid and base conditions was termed the fulvic acid portion. As these two fractions were studied in greater detail, it was found that the humic acids were larger organic molecules (between 2,000 and 10,000 daltons) and the fulvic acids were smaller (between 500 and 2,000 daltons) (6).

Two proposed structures for humics are available in the literature (5). Although the models are not identical, they are predominated by polymers of aromatic rings with hydroxyl groups and carboxylic groups. In 1975, Morris (7) proposed a reaction mechanism for the formation of trihalomethanes that has subsequently been termed the haloform reaction.

This mechanism is shown in Figure 2. Working with model precursors, Rook (5) proposed the second major mechanism where the main point of attack by chlorine was one of the meta-positioned hydroxyl groups on an aromatic ring (Figure 3). It is noteworthy that both these reaction mechanisms explain why THM formation is enhanced under higher pH conditions.

Algal ECP

In 1980, the precursor pool was expanded to include ECP from algae (8,9,10). The excretion of organic products was first well defined in the late 1950's and the early 1960's (11-19). These authors demonstrated that several forms of organic carbon were released by algae as a result of photosynthetic activity. The amount of carbon released has been expressed as a percent of the total carbon fixed and has been found to range between two and 70 percent (20). The molecules released vary in size from simple organic sugars (21) to high molecular weight compounds (20).

The nature of ECP has been of interest to aquatic biologists for some time because up to 80 percent of the excreted organic materials can be used by heterotrophs (22), making ECP a significant factor in both quantifying primary production and determining trophic relationships in natural waters. Two strategies have been taken by investigators to try to characterize the organic compounds released as ECP. These

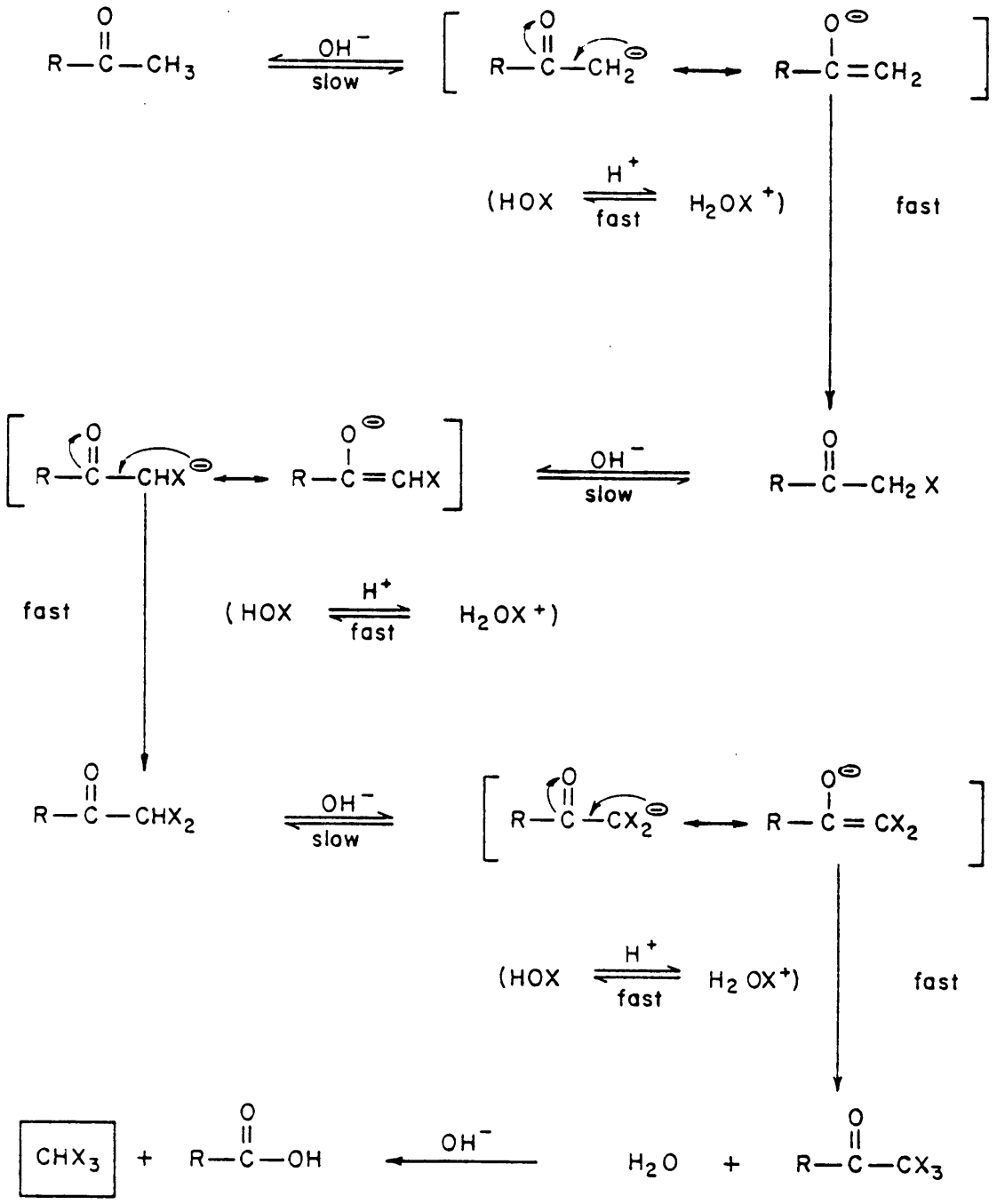


Figure 2. Haloform reaction proposed by Morris (7).

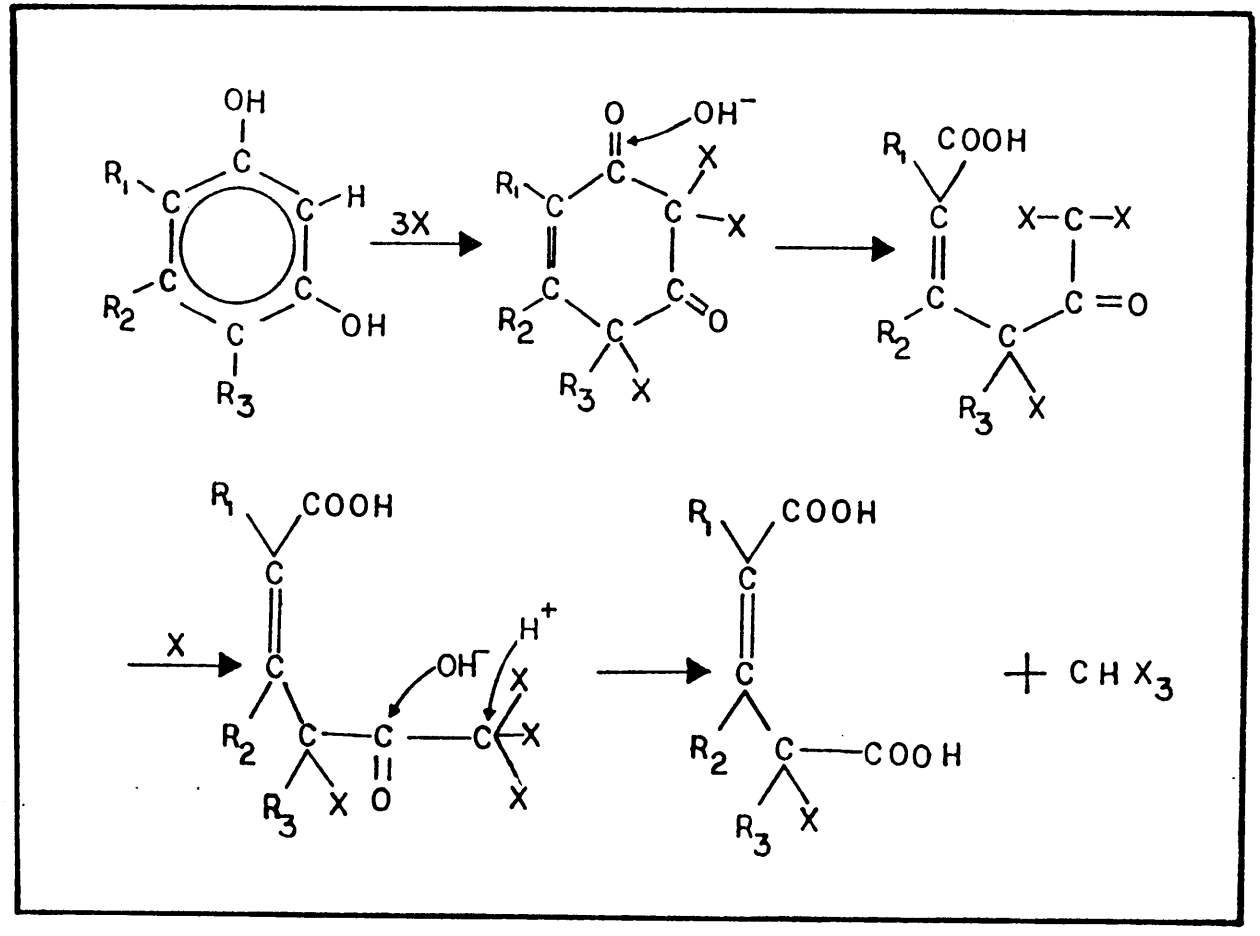


Figure 3. Rook's reaction mechanism between chlorine and humic acids from reference 5. Note: X represents any haloform.

are: 1) to identify the particular organic molecules released (21) and 2) to characterize them on the basis of molecular weight (20). While the first approach reveals significantly more about ECP, it is much more rigorous and, for the purposes of environmental engineering, is not entirely necessary. Molecular weight, however, has been found to be a significant factor effecting the removal efficiency of organic matter during water treatment and, thus, is important to environmental engineering (23).

Size Fractionation of THM-precursors

Recent research on coagulation of drinking water supplies has centered on THM-precursor removal. Sinsabaugh (23) found that, among other parameters, the size distribution of the organic pool was an important factor in how efficiently the organics were removed by coagulation. Thus, the organic size distribution may effect the THM-precursor removal efficiency of alum coagulation. For this reason the molecular weight distribution of naturally occurring organic substances is of more than academic concern.

Humic compounds (humic and fulvic acids), as well as algal ECP, have been fractionated according to size. Oliver and Visser (27) extracted humic and fulvic acids from two sources, a lake and a stream, for size fractionation. In a similar effort to characterize organic substances of a specific origin, Chrost and Faust (20) fractionated ECP from

cultured algae. In addition, several studies (23-26) have dealt with the molecular size distribution of entire organic pools. The data from all these studies are presented in Figure 4.

The pattern of the individual MW distributions can be scrutinized to reveal information regarding the distribution of the organics with respect to size. Because the data have been plotted in a cumulative fashion, the slope of the curve, not the height, reveals the amount of organics present in a size range. The steeper the slope, the more organics present. A good example of this was provided by four distributions of humic substances which follow similar patterns. The cumulative distribution curves of the two fulvic acids show a small slope from the begining to about the 1K dalton size. The slope is about double from 1 to 20 Kd, and above 20 Kd, the curve is flat indicating fewer organics in this size range.

A similar analysis of the humic acid curves indicate that they range from 10 Kd to about 100 Kd (and even greater for the stream humic acids). These data are compared to those from Thurman (6) in Table 1. As can be seen a disparity exists between the two sources as to the true size of humic and fulvic acids.

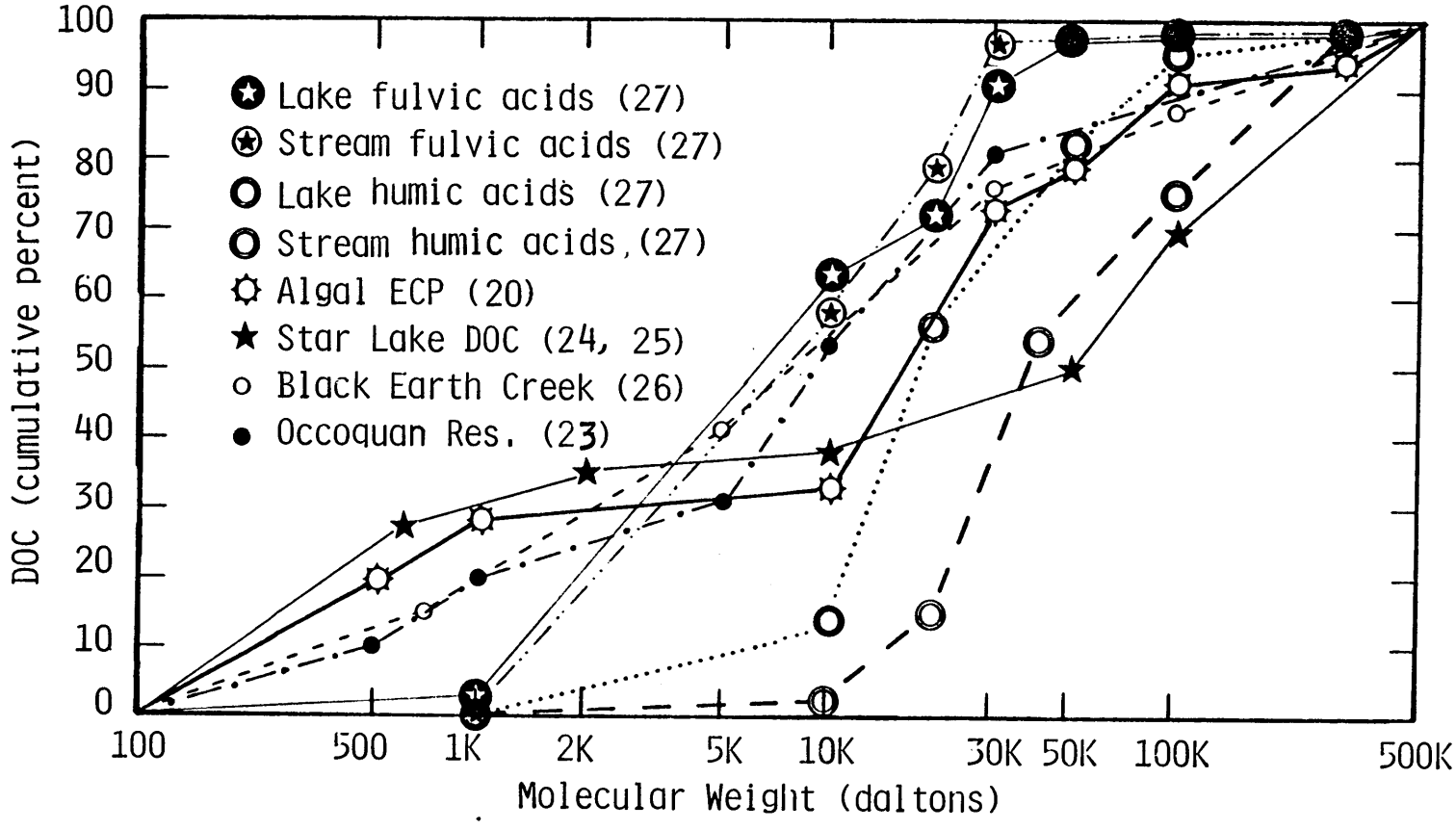


Figure 4. Molecular size distributions for various organic pools.

Table 1.
A comparison of humic and fulvic acid size ranges from different sources.

Source	humic/fulvic acid	MW* (Kdaltons)	Method	Reference
Lake Water	fulvic	0.5 - 2	1	6
	humic	---	1	6
	fulvic	1 - 30	2	27
	humic	10 - 100	2	27
Rivers & Streams	fulvic	0.5 - 2	1	6
	humic	2	1	6
	fulvic	1 - 30	2	27
	humic	10 - 300	2	27

*MW = molecular weight
1 = small angle X-ray scattering
2 = ultrafiltration

THM Yield

Organic substances in water supplies have also been characterized by their ability to form THMs upon chlorination. Rook (3) coined the term "yield" to describe this tendency and expressed it as μmoles $CHCl_3$/μmoles DOC on a percent basis. A typical yield for humic acids is in the range of 0.5-1.0 μmoles $CHCl_3$/μmoles DOC. The precise yield is, of course, dependent on the functional groups present and the structure of the organic molecule. ECP yields of THMs cover a wider range (0.02-4.0 μmoles $CHCl_3$/μmole DOC percent) (8) but are, in general, greater than those from humics. The presence of ECP in surface waters is a function of the species of algae present, diurnal variations, seasonal variations, and bacterial activity. Up to this point, no proposed reaction mechanism for the formation of THMs from ECP has been suggested, but it is probably similar to that which occurs upon the chlorination of humic acids.

Hydrilla verticulatta

Hydrilla is a submerged aquatic macrophyte that has gained notice recently because of its rapid infestation in lakes of the southeast region of the United States. This plant was probably introduced to this country when it was imported as an aquarium plant. As early as the latter 1950's it was found growing in the wild in the lakes of Florida. Now more than one-half million acres (2×10^9 m^2) of Florida's surface wa-

ters are inundated with hydrilla. Currently, the plant is spreading as far west as California and as far north as Iowa in the Mississippi basin. In Virginia, hydrilla was first found in the Potomac River in 1982. Soon, this weed had spread to many nearby impoundments (28).

Some experts believe that the appearance of hydrilla is a good sign for the reservoir. The proponents of the "hydrilla is good" concept say 1) that it provides refuge for fish food, so fishing improves, and 2) that its reappearance in the Potomac River has signalled improved water quality.

Other experts believe hydrilla's detrimental effects far outweigh its benefits. The problems associated with hydrilla stem from its rapid growth rates. Once established, hydrilla is quite prolific making it difficult to control. Some of the traditional problems include the following: 1) choking off of waterways to recreation and commerce, 2) serving as a breeding ground for mosquitoes, and 3) disrupting the existing ecosystem. Recent technology has born a new concern over hydrilla, that being the potential for hydrilla to add to the THMFP of the waters it inhabits.

The Potential For Problems In the Occoquan Reservoir

Before hydrilla can pose problems in the Occoquan Reservoir, it must become established in sufficient quantities. A literature search revealed that although hydrilla can adapt to almost any type of environment, there are optimal condi-

tions regarding nutrient levels, sediment type, climate and light availability.

Nutrient Levels

Optimum nutrient and mineral levels for rapid growth are presented in Table 2. More recent data (30) indicate that 1) increases in potassium stimulate increased growth rates; 2) increases in calcium are associated with decreases in growth rate; and 3) phosphorus concentrations are limiting below 1.6 ug/L.

Sediment Type

Sediment type was the next parameter evaluated. Bruner and Batterson (31) found that both faster growth and enhanced tuber production were experienced when hydrilla were raised in marl rather than sand or potting mix. Tubers, a form of vegetative propagule, can serve as a source of regrowth in areas in which above-ground material has been controlled by chemical or mechanical means. Table 3 compares the sediment fertility of the Occoquan Reservoir to that found for the potting mix and marl used by Bruner and Batterson (31).

Climate

Hydrilla's presence both in the Potomac River and in Iowa indicates that the temperatures in more northern regions can be tolerated by the plant. However, it is not known if cold

Table 2.
Mineral concentrations for optimum hydrilla growth. Data from references 29 and 30.

Element	Concentration (mg/L)
Ca^{+2}*	10.0
N^{+5}	2.1-21
K^{+1}*	35.0
Mg^{+2}	0.50-5.0
S^{-6}	0.60-6.0
P^{-5}*	0.002
B^{+3}	0.005-0.05
Mn^{+2}	0.06
Cl^{-}	0.11
Cu^{+2}	0.02
Mo^{+6}	0.001
Zn^{+2}	0.005
Fe^{+2}	0.01

*These data are from reference 30.

Table 3.
Sediment fertility of the Occoquan Reservoir compared to that of the potting mix and marl used by Bruner and Batterson (31)

	Potting mix (31)	Marl (optimum) (31)	Occoquan (32)
organic matter (mg/g-dry)	94	28	62-87
N (mg/g-dry)	3.1	1.4	1.9-2.4
P (mg/g-dry)	0.5	0.3	0.4-1.3
K (mg/g-dry)	0.07	0.14	no data

will limit its rate of growth. Although hydrilla will grow in these climates, it may not proliferate to the extent that it does in warmer climates. To make this determination, it would be revealing to see if hydrilla biomass per unit area decreases in lakes located at increasing latitudes. Data of this type were available from two studies in which hydrilla density was measured in four Florida lakes (33,34). Figure 5 shows that decreases in biomass occur with respect to latitude. The values that might be expected for the Occoquan are indicated by the dashed lines. It must be emphasized that these estimations were based on data from two studies and that the differences between these lakes may be due to any one of the other parameters listed previously.

Light Availability

Light availability is probably the most common limiting factor in plant growth. In terrestial ecosystems, taller plants often shade the shorter ones, which must either adapt to low light conditions or find an appropriate opening. In aquatic ecosystems, however, the turbidity of the water is often an additional factor responsible for restricting light. Although the light requirements for hydrilla were not available, it is assumed that it has requirements similar to that of most aquatic macrophytes. The Wisconsin Department of Natural Resources uses an equation that, based on Secchi disk transparency values, predicts the depth at which an aquatic

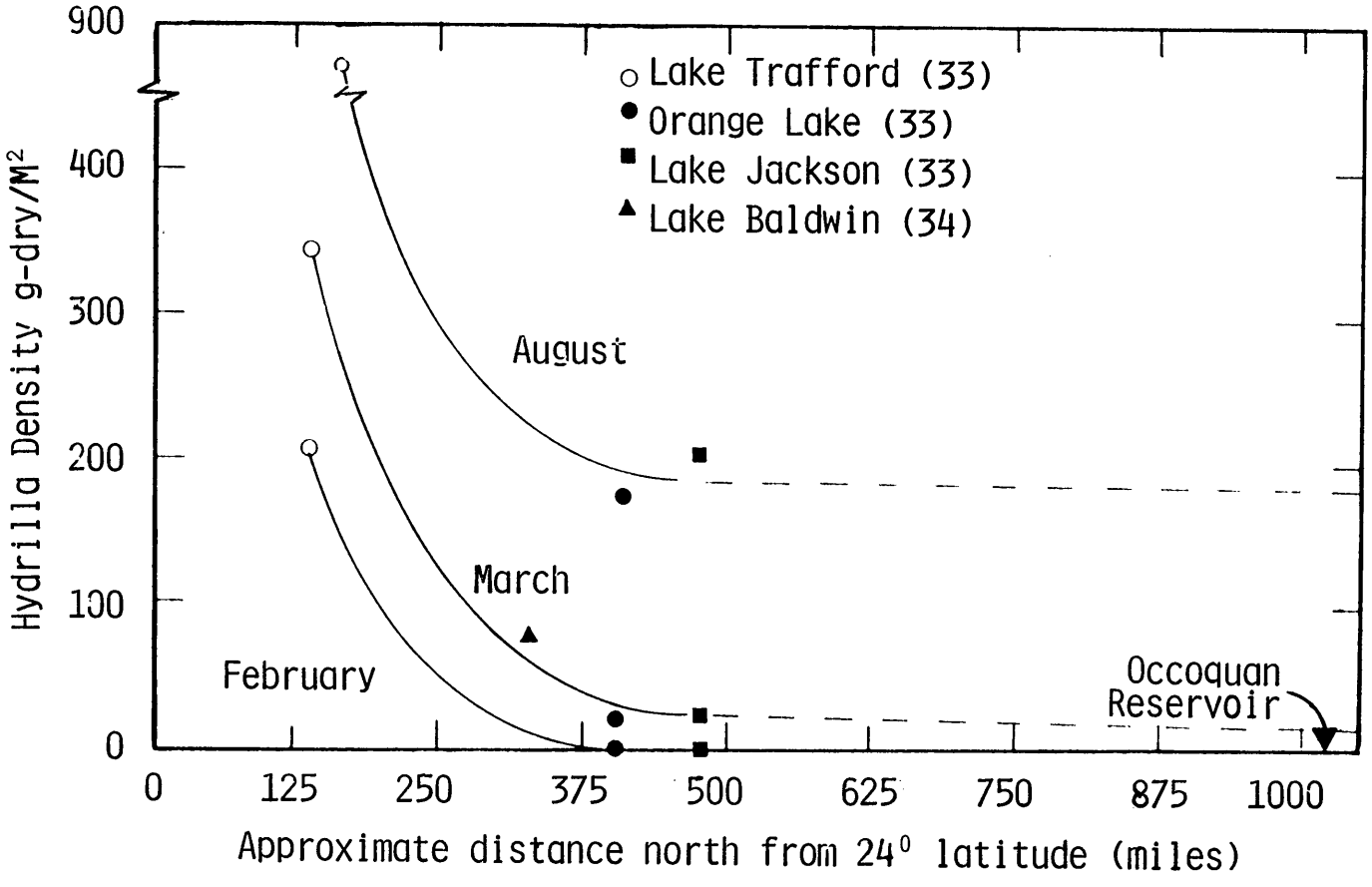


Figure 5. Estimation of hydrilla density in the Occoquan Reservoir.

macrophytes community can be expected to grow. This equation is easy to use because Secchi disk transparencies are common in the literature, and many data of this type are available for the Occoquan Reservior. The relationship between Secchi disk transparency values and maximum depth of plant growth is as follows:

$$Y = 0.83 + 1.22X$$

where:

Y = maximum depth of plant growth (meters)

X = Secchi disk depth (meters)

Although the summer Secchi disk depth in the Occoquan Reservoir is normally less than one meter, a value of 1.25 meters is used in calculations to simulate a worst case scenario. Thus:

$$Y = 0.83 + (1.22 \times 1.25)$$

$$Y = 2.4 \text{ meters}$$

A hipsographic curve (Appendix A) was used to provide an estimate of the lake area covered by 2.4 meters of water or less. The analysis showed that 2.4 million m^2 (about 30 percent of the lake area) would support hydrilla. Again to simulate worst case conditions, a value in excess of that which is predicted by Figure 2 was used (400 grams hydrilla-dry/m^2 of lake bottom). The product of these data (2.4 X $10^6 m^2$ X 400 g-dry/m^2) can be used to estimate the hydrilla biomass that might be expected to inhabit the Occoquan should

it become established (9.6×10^5 kg). The THMFP data generated from this study could then be integrated to predict the overall increase in THMFP that may occur if hydrilla did inhabit the Occoquan Reservoir.

CHAPTER III

METHODS

Overview

This chapter outlines the methods used in the experiments described below. Four experiments were performed to determine the THM-precursor release by hydrilla. The first one explored the effect sediment had on the THM-precursor production by hydrilla. THMFP from the media supporting hydrilla raised in the presence of sediment was compared to that from hydrilla raised with no sediment present.

The second experiment investigated the effect of light on THM-precursor production. Ehrlynmeyer flasks containing hydrilla sprigs in a nutrient solution were split into two groups. One group was subjected to a 12 hour light/12 hour dark diurnal cycle for 60 hours and the other group was placed in the dark for the same time period. After this incubation period, the THMFP of the nutrient solutions was determined.

The third experiment quantified the amount of THM-precursors released by decaying hydrilla. Hydrilla was placed in a solution of copper sulfate and left in the dark until it died. THMFP was then monitored as the plant decayed.

In the fourth experiment, the effect of turbulence on precursor production was investigated. The plant was placed in a nutrient solution which was constantly agitated by a paddle stirrer. The THMFP of the supporting media was then monitored periodically for two weeks.

In addition to the above four experiments, the organics released by hydrilla (both as it grew and as it decayed) were size fractioned by ultrafiltration.

Sample Collection

In late October, 1984, hydrilla from the Potomac River and lake water and sediment from the Occoquan Reservoir were collected by Virginia Tech staff and returned to the campus laboratory facilities. The sediment and water samples were placed in aquaria that served to culture the hydrilla plants that were subsequently used in experiments. The plants were exposed to alternating periods of light and dark (12 hours each) at a temperature of 23° C. Plants were periodically removed for use in experiments.

Analytical Techniques

In all four experiments, four water-quality characteristics were determined. These were: pH, alkalinity, DOC and THMFP. Alkalinity and pH were measured simultaneously by methods described in Standard Methods (35) on a Corning model 610-A pH meter. Organic carbon concentrations were deter-

mined by analysis with a Dohrmann/Envirotech (Santa Clara, California) model DC-54 ultra-low level organic carbon analyzer. Replicate measurements were within two percent.

Determinations of THMFP were performed as follows. Water samples were chlorinated at a chlorine : DOC weight ratio of 3:1 and incubated in the dark at 22° C. The solution was buffered to pH 7.0 with pH-7 phosphate buffer (35). The samples were incubated for seven days before THMFP determinations were made. If it were not possible to analyze the samples after exactly seven days, THM formation reactions were terminated by the addition of sodium sulfite to reduce chlorine, and the pH was depressed to pH 2 by the addition of nitric acid to prevent the hydrolysis of THMs from organic halide intermediates. The quenched samples were refrigerated until they could be analyzed.

The THM's were determined by the purge-and-trap method described in Section 514 of Standard Methods (35). A Tracor (Austin, Texas) model 560 gas chromatograph equipped with both a Tracor LSC-2 purge-and-trap module and a Hall electrolytic conductivity detector was used.

Experimental Procedures

Experiment I

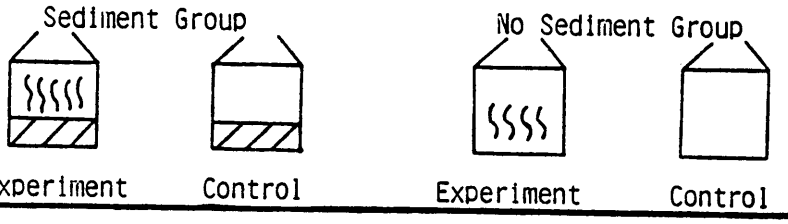
Hydrilla was acclimated to the laboratory environment before experiments commenced. As described in the overview,

four different experiments were performed (Figure 6). In the first experiment, hydrilla was placed in two large containers, one with sediment, the other without. In addition, two containers which did not contain hydrilla served as controls. As with the experimental vessels, one of the controls contained sediment and the other did not. The jars were placed in an incubator which maintained a 12 hour light/12 hour dark cycle and a temperature of 23°C. Samples were withdrawn periodically, filtered with a Gelman A/E glass-fiber filter to exclude particulates, and then analyzed for pH, alkalinity, DOC, and THMFP.

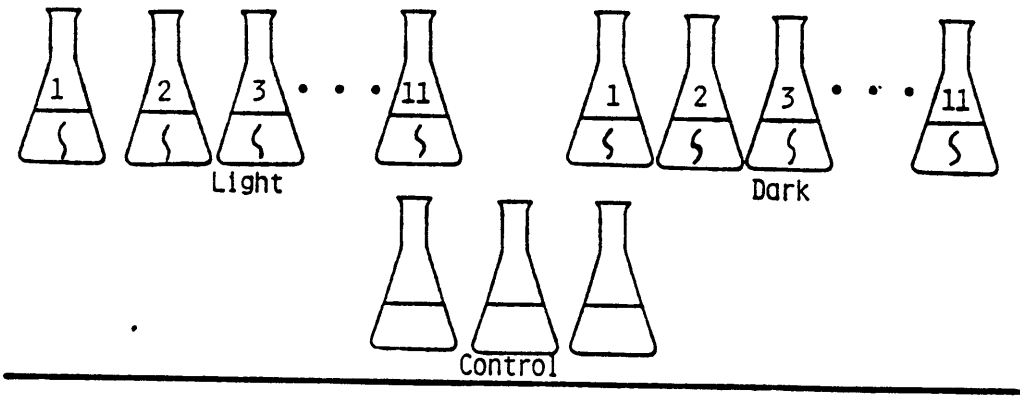
Experiment II

The second experiment was designed to quantify the extent of THM-precursor formation by hydrilla during photosynthesis. Sprigs of hydrilla were weighed and then placed in twenty two 250-mL Erlynmeyer flasks containing 200 mL of nutrient solution (Appendix B). Eleven of these were exposed to light for a 12 hour light/12 hour dark diurnal cycle for 60 hours, and the other eleven were left in the dark for 60 hours. In addition, three flasks containing only the 200-mL of nutrient solution were allowed to incubate as controls. At the end of the 60-hour period, the plants were removed from the flasks, dried overnight at 105° C, and reweighed. Wet weight and dry weight comparisons are listed in Appendix C.

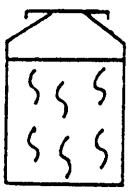
Experiment I. Effects of Sediment on THM-Precursor Production by Hydrilla



Experiment II. Effects of Light on THM-Precursor Production by Hydrilla



Experiment III. THM-Precursor Production by Decaying Hydrilla



Experiment IV. Effects of Water Movement on THM-Precursor Production

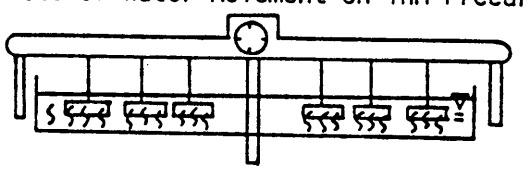


Figure 6. Four experiments used in this study.

ζ = hydrilla sprigs

Aliquots of the nutrient solution were removed from the flasks and analyzed for pH, alkalinity, DOC and THMFP.

Experiment III

The third experiment was designed to determine the extent of THM-precursor production during the decay of hydrilla. Initially, sprigs of hydrilla were heat-shocked in boiling water and allowed to decay. Occoquan Reservoir sediment from the aquaria was then introduced as a source of microbes to promote decay. This technique proved infeasible because the THMFP induced by the sediment was much greater than the THMFP of the decay products from the plants themselves. To avoid the necessity of inoculation, hydrilla was placed in a solution of copper sulfate and left in the dark until the plant began to decay. The decaying mass was removed from the copper sulfate solution, rinsed with distilled water, blotted dry, and placed in 2.5 L of organic free (Milli-Q) water. It was necessary to wash the hydrilla because a precipitate formed upon chlorination when copper sulfate was present. To prevent both the possible regrowth of hydrilla and the development of an algal population, the decaying mass was kept in the dark. Both DOC and THMFP were determined periodically.

Experiment IV

In many seemingly quiescent lakes there are unseen currents and wave actions that may have a profound influence on the biota; therefore the fourth experiment was designed to account for this possibility. The hydrilla was placed in a long trough and the supporting media was constantly agitated by paddle stirrers. As in the other experiments, the hydrilla was exposed to a 12 hour light/12 hour dark diurnal cycle at a temperature of 23°C. Samples were withdrawn over a period of thirteen days and analyzed for pH, alkalinity, DOC and THMFP.

Size-Fractionation of Organic Carbon

Two sources of organic materials derived from hydrilla were size fractionated: those from decaying hydrilla and those from growing hydrilla. To avoid contamination, the hydrilla were washed extensively with distilled water to remove aquarium water, sediment, and algae. The hydrilla was then placed in organic-free nutrient mix until enough DOC was present to allow for adequate measurement (about 1.5 weeks). The solution used for the fractionation of organics from decaying hydrilla came from Experiment III (Figure 6).

Organic matter was fractionated into size catagories by first excluding particulate organic carbon (POC) with precombusted Gelman type A/E glass-fiber filters. The filtrate was then filtered through three Amicon series YM and one se-

ries YC ultrafilters. Table 4 lists the filters used along with their appropriate pore sizes and molecular exclusion limits.

The ultrafilters were stored in ten percent ethanol at 4°C to prevent bacterial growth on the membrane. Prior to use, the ethanol was removed from the filters using a two step procedure. First, the filters were placed in about 200 mL of distilled water for one or two days to leach out most of the ethanol. It was necessary to change the distilled water periodically to achieve adequate ethanol removal. Second, the filters were mounted in the ultrafiltration cells and flushed with organic free water. When the DOC concentration of the filtered organic free water was equal to its original value, the ultracell was prepared for the filtration of the sample by rinsing it out twice with the water that was to be filtered.

Ultrafiltration was performed in a stirred, 200-mL Amicon ultracell under a pressure of 2.8 kg/cm^2 nitrogen at a temperature of 25°C. After fractionation, the organic carbon concentrations in bulk water and filtrates were determined. A subsample was also collected for chlorination and subsequent analysis for THMFP.

Table 4.
Pore size and nominal molecular-weight exclution limit of ultrafilters.[1]

Membrane	Approximate pore size (nm)	Nominal molecular-weight exclusion limit (daltons)
YC05	1.1	500
YM5	1.5	5,000
YM10	1.6	10,000
YM30	2.1	30,000

[1]Data from Amicon Co., Lexington, MA.

CHAPTER IV

RESULTS

Ability For Growth In The Occoquan Reservoir

Table 5 compares the mineral concentrations of the Occoquan Reservoir to the optimum concentrations found in Table 2. For the most part, the Occoquan had sufficient quantities of these minerals. Exceptions, however, included potassium, which was about one-tenth the optimum concentration, plus manganese and copper which were both below detection in the water column. Although no data regarding the potassium content of Occoquan sediment exists (Table 3), it was suspected that this mineral was present in sufficient quantities in the sediment to permit hydrilla colonization of the Occoquan Reservoir. This contention was supported by the fact that the hydrilla did survive in all three of the laboratory aquaria containing sediment and water from the Occoquan Reservoir.

Table 6 indicates that the DOC concentrations were much greater in jars containing sediment than in those without it, both in the experimental and in the control groups. Most likely, organic matter in the sediment was released into solution. The contribution of hydrilla to the DOC pool was determined by subtracting the control from the experimental

Table 5.
Mineral concentration for optimum hydrilla growth compared to the mineral concentrations in the Occoquan.

Element	Concentration (mg/L) Optimum	Occoquan
Ca^{+2}	10.0	17.0
N^{+5}	2.1-21	0.40
K^{+1}	35.0	3.7
Mg^{+2}	0.50-5.0	4.3
S^{-6}	0.60-6.0	8.3
P^{-5}	0.002	0.07
B^{+3}	0.005-0.05	ND
Mn^{+2}	0.06	≤0.01
Cl^{-1}	0.11	16.2
Cu^{+2}	0.02	≤0.005
Mo^{+6}	0.001	ND
Zn^{+2}	0.005	0.01
Fe^{+2}	0.01	0.13

Note: The values reported for N and S include only nitrate and sulfate respectively.
ND = no data

Table 6.
Experiment I: THM-precursor production by quiescent hydrilla in microcosms with and without Occoquan Reservoir Sediment.

Group I, hydrilla with sediment

Mass = 39.473 grams-wet, 2.353 grams-dry

DAY	D/N $	DOC (mg/L) exp*	DOC (mg/L) cont@	THMFP (ug/L) exp	THMFP (ug/L) cont	DOC mg/g-dry	THMFP ug/g-dry
0	D	5.96	6.05	101	120	0.00	0.0
1.75	N	7.13	7.18	128	98	0.00	30.2
2.50	N	5.35	5.85	120	61	0.00	72.1
4.00	D	6.03	6.01	112	56	0.02	55.4
4.70	N	4.13	4.07	112	69	0.06	42.0
5.00	D	4.15	4.13	173	80	0.01	97.0
10.00	D	4.08	5.34	158	86	0.00	82.2

Group II hydrilla without sediment

Mass = 37.369 grams-wet, 2.230 grams-dry

DAY	D/N $	DOC (mg/L) exp*	DOC (mg/L) cont@	THMFP (ug/L) exp	THMFP (ug/L) cont	DOC mg/g-dry	THMFP ug/g-dry
0	D	4.25	4.19	7	3	0.06	5.0
1.75	N	5.96	5.85	9	19	0.11	0.0
2.50	N	5.28	5.18	35	4	0.10	33.6
4.00	D	4.70	4.54	5	2	0.16	5.0
4.70	N	4.11	4.06	36	2	0.05	35.2
10.00	D	5.13	5.30	55	5	0.00	58.7

$Day/Night
*Experimental group, nutrient mix (2.5 L) and hydrilla
@Control group, nutrient mix (2.5 L) alone

group and normalizing the remaining concentration by expressing it as a function of the dry weight of the plant. When expressed in this manner, the data indicated that hydrilla made no appreciable contribution to the organic pool in neither the jar with sediment nor the one without it during the days of this study.

Sediment Effects On THM-Precursor Production: Experiment I

Figure 7 shows how the THMFP varied with time. The curves presented were developed using the lines of best fit for curvilinear regressions. Although it was quite variable, the THMFP of the solution with hydrilla in the presence of sediment increased exponentially for about three days and then started to level. The THMFP of the solution with only hydrilla (no sediment) was still increasing exponentially through day ten, but at a much lower rate than that of the solution containing hydrilla in the presence of sediment. The maximum THM concentration developed from organic matter in the flasks containing sediment was about 82 ug per gram dry weight (ug/g-dry); in those without sediment, the maximum concentration was 59 ug/g-dry. The higher THMFP associated with hydrilla cultured with sediment did not result from the leaching of organic matter from the sediment alone, as this source was discounted by subracting sediment-released THM precursors from the experimental group. This observation

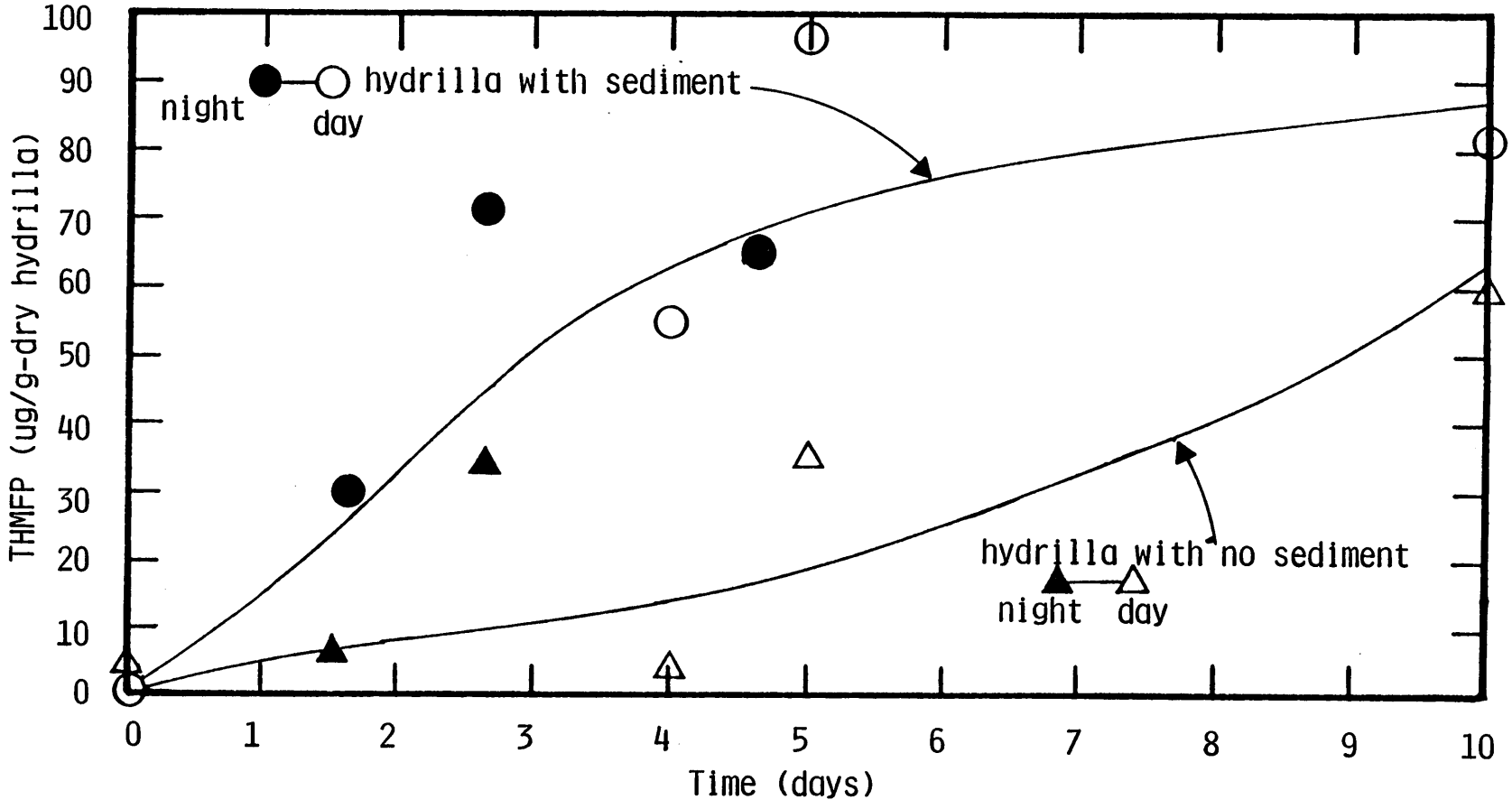


Figure 7. Change in trihalomethane formation potential (THMFP) through time with attention to differences between light/dark cycle.

suggests that the sediment was affecting the production of THM precursors by the hydrilla in some manner.

Attention was given to the time of day that each sample was taken prior to analysis to determine whether there was any diurnal variation in the production of THM-precursors. Samples taken during the day are designated in Figure 7 by open symbols and those taken during the night with darkened ones. As can be seen, no pattern that would indicate a diurnal cycle was evident in either of the test cultures.

Light/Dark Experiments: Experiment II

This series of experiments was performed to explore further the effect of light on THM-precursor production by hydrilla. Sprigs were placed in 250-mL Erlynmeyer flasks and then segregated into two groups. One group was placed in the dark for 12 hours and the other group was placed under a plant light for 12 hours. Three flasks containing only nutrient mix (no plants) were used as controls. Table 7 shows that in comparison to the results of the previous experiment, the DOC concentratons were quite high, whereas the THMFP levels were equivalent.

There was little difference in the amount of DOC released by plants exposed to the light and that released by those kept in the dark. Although the THMFP showed a difference between the light and dark conditions, they were quite vari-

Table 7.
Experiment II: DOC and THMFP from hydrilla sprigs in 200 mL of media for 12 hours.

LIGHT

Dry-weight (grams)	DOC (mg/L)	THMFP (ug/L)	DOC (mg/g-dry)	THMFP (ug/g-dry)
0.118	0.513	28	0.15	13.8
0.116	0.503	29	0.23	24.1
0.147	0.507	56	0.19	55.8
0.085	0.423	25	0.13	23.5
0.060	0.416	28	0.16	43.3
0.066	0.510	36	0.43	63.6
0.047	0.432	33	0.27	76.6
0.052	0.457	35	0.35	80.0
0.037	0.427	23	0.31	43.2
0.090*	0.479	23	0.18	13.3
0.031*	0.463	18	0.45	12.6
	mean values		0.26	40.9 ±17.0

DARK

Dry-weight	DOC	THMFP	DOC	THMFP
0.195	0.670	33	0.31	18.5
0.044	0.289	23	0.00	36.4
0.078	0.439	31	0.18	41.0
0.070	0.564	36	0.55	60.0
0.147	0.570	54	0.27	53.1
0.052	0.422	22	0.20	26.9
0.099	0.611	18	0.49	6.1
0.032	0.405	22	0.23	43.8
0.044	0.489	15	0.55	00.0
0.110*	0.400	25	0.04	18.2
0.031*	0.455	15	0.42	00.0
	mean values		0.29	27.6 ±14.1

*Samples in 150 mL of media.

CONTROLS
(means of three samples)
DOC = 0.369 (mg/L)
THMFP = 15 (ug/L)

able. Statistical analysis (t-test) revealed that the data sets were not different at the 95 percent level of confidence.

Contributions Of THM-Precursors By Decaying Hydrilla: Experiment III

The decaying plants released more organic matter, including THM precursors, than did live plants. Figure 8 shows that both DOC and THMFP increased as the decay process continued, especially beyond day eight. The increase in THMFP was greater than the increase in DOC.

Effect Of Water Movement On THM-Precursor Production: Experiment IV

Turbulence appeared to have a significant effect on the release of both DOC and THM precursors. First, both THM precursors and DOC were released from the hydrilla in a stirred microcosm (Figure 9) in much greater quantities than from the hydrilla in a quiescent setting (Figure 7). In addition, Figure 9 shows that hydrilla in the stirred microcosm released DOC and THM-precursors at a steady rate without the oscillation found in a quiescent setting (Figure 7).

In all four experiments, the alkalinity of the nutrient mix never varied from the initial 40 mg/L as $CaCO_3$. Likewise, the pH remained within one-tenth pH units of 7.0 at all times during all experiments.

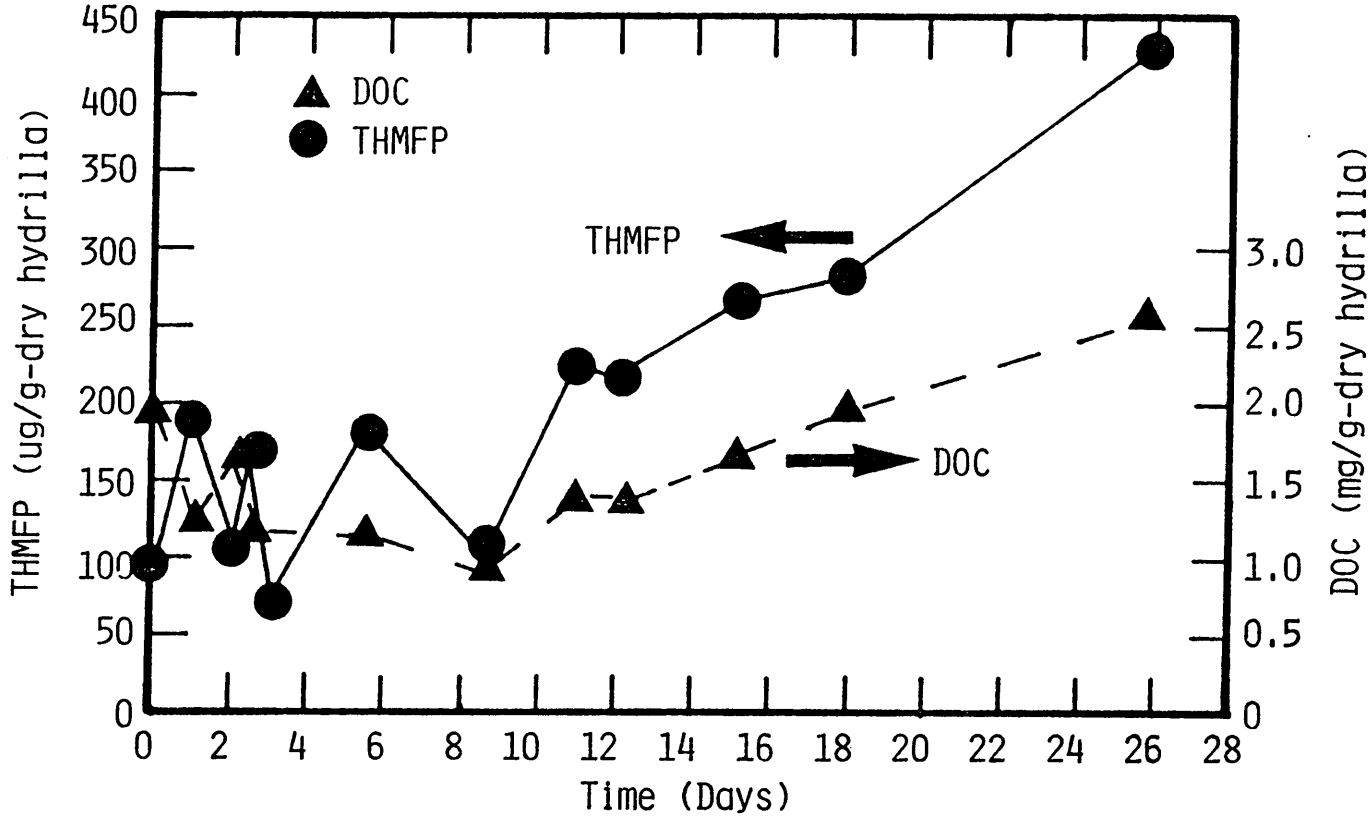


Figure 8. Trihalomethane formation potential (THMFP) and dissolved organic carbon (DOC) concentration of solution containing decaying hydrilla.

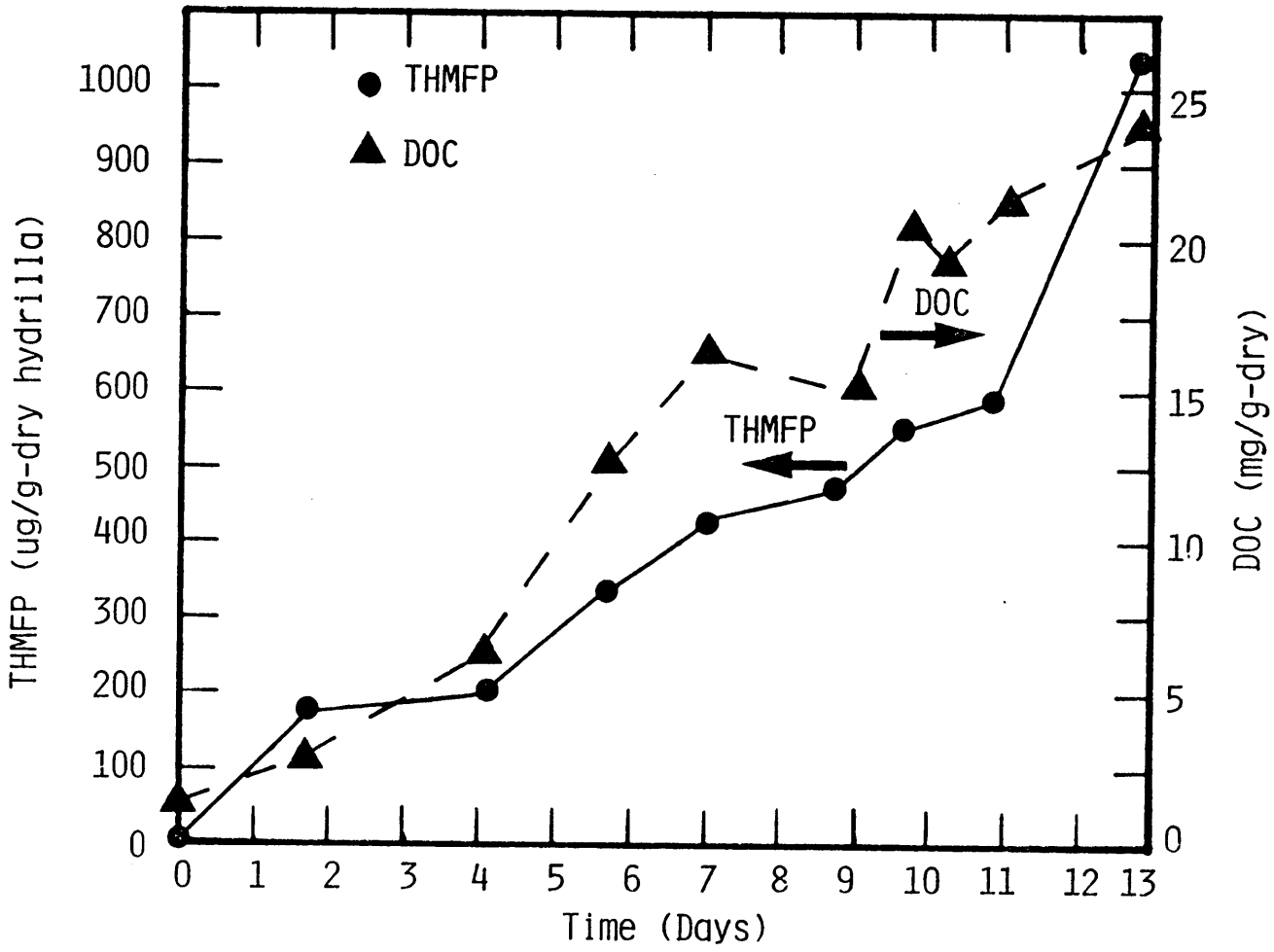


Figure 9. Trihalomethane formation potential (THMFP) and dissolved organic carbon (DOC) concentrations in stirred microcosm containing hydrilla suspended in nutrient medium

Size Fractionation

Arrangement of graphs

Figures 10 through 15 illustrate the DOC, THMFP, and yield for the five different size fractions. The data are presented in a recurring series of three graphs as follows: 1) growing hydrilla graphed in bar-chart form, 2) decaying hydrilla, again in bar-chart form and 3) a comparison of the organics released by growing and decaying hydrilla to each other in straight line form (showing cumulative data). In all of these size fractionation graphs, the molecular sizes were presented on a logrithmic scale.

Molecular size distribution

Figure 10A shows the size distribution of organics released by growing hydrilla. The total DOC concentrations were quite low. This was expected in light of the DOC released by growing hydrilla observed in previous experiments. Only two size fractions were present in the waters containing growing hydrilla. Most (88 percent) of the organic molecules were less than 500 daltons and the remaining 12 percent were greater than 30,000 daltons. In general, the size of the organic molecules from growing hydrilla were quite small.

Figure 10B showed the distribution of organic molecules in waters containing decaying hydrilla. In contrast to the organic molecules released by growing hydrilla, the organic

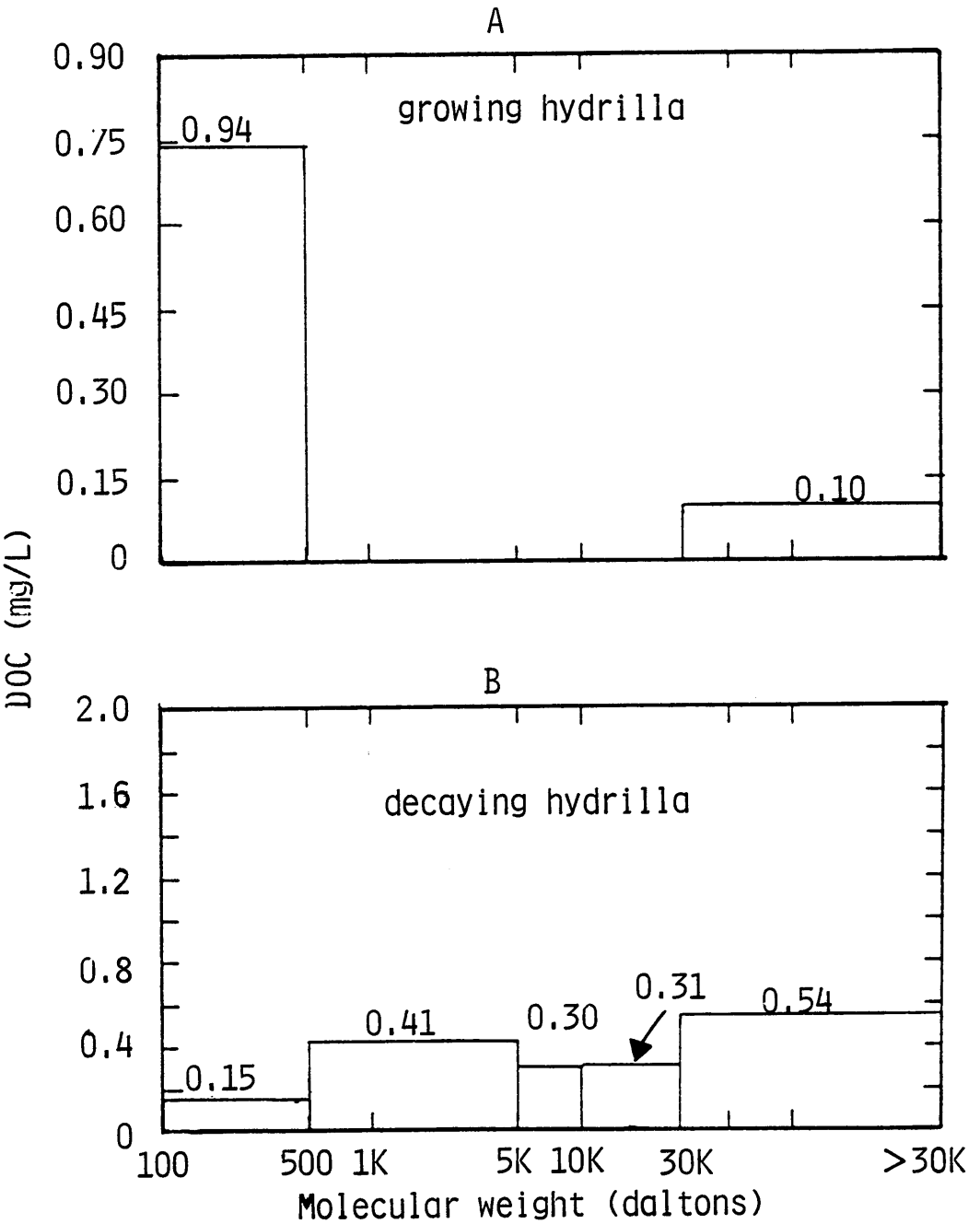


Figure 10. Size distribution of organics released by growing hydrilla (top) and by decaying hydrilla (bottom).

molecules released by decaying hydrilla were quite well distributed throughout the size ranges studied. However, 32 percent (0.54 mg/L) of the total organic pool was composed of organic molecules greater than the largest filter size (30 Kd). This was unfortunate because it was not possible to further characterize these large organic molecules.

Figure 11 compares the MW distributions of the DOC released by growing hydrilla and decaying hydrilla to that of a reference fulvic acid extracted from the Swannee Stream (dashed line). The Suwannee Stream fulvic acid has been well characterized on the basis of molecular weight and is thus made available by the International Humic Substances Society's (IHSS) Standard Reference Committee (5293 Ward Road, Arvada, CO 80002) for use as an analytical standard. In this study, the IHSS fulvic acid served to 1) evaluate the accuracy of the ultrafilters, and 2) provide a basis of comparison for the molecular size of the organic compounds from hydrilla. The IHSS reference fulvic acid is reported to be composed of compounds with molecular weights less than 2K daltons. Figure 11 shows that the molecular-weight determined by the ultrafilters used in this study agreed as closely as possible with the one reported (ie. 100 percent of the reference fulvic passed the 5K dalton membrane). Because an ultrafilter with an exclusion limit of 2K daltons was not used, it was not possible to acheive an exact agreement with the reported molecular size of less than 2K

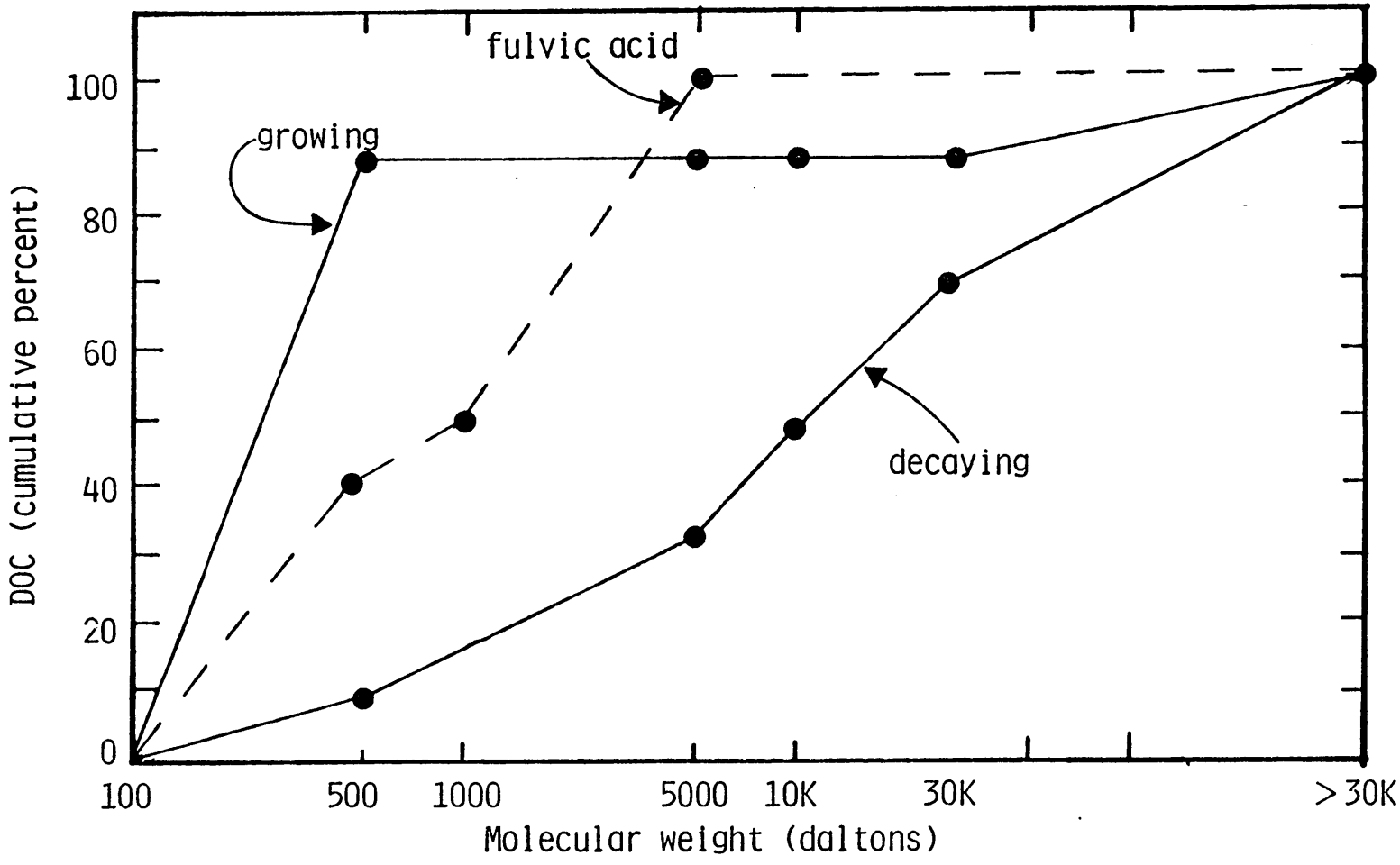


Figure 11. A comparison of the cumulative percentages of dissolved organic matter (DOC) among the various molecular-weight distributions for organics released by both growing and decaying hydrilla and the reference Suwannee Stream fulvic acid.

daltons. With the exception of increased quantities of organic molecules in the small size range, the MW distribution of the organic molecules released by decaying hydrilla was quite similar to those found in the literature for naturally occurring organic compounds (Figure 4). The size distribution of organic compounds from the solution containing growing hydrilla was quite different than most naturally occurring organic compounds (Figure 4) in that the organic compounds from growing hydrilla were much smaller, 88 percent of them being smaller than the smallest filter (500 daltons exclusion limit). Overall, the organic compounds released by decaying hydrilla were composed of the largest organic molecules, followed by the organic molecules released by growing hydrilla and the reference fulvic acids.

Size distribution of THMFP

Figure 12A shows the THMFP of the five organic size fractions produced by growing hydrilla. The size distribution of the THM-precursors differed appreciably from that of the parent organic pool (Figure 10A). As shown, the extremes (large and small) of the size distribution produced most of the THMs with decreasing THM concentrations towards the middle size range. This may be expected by virtue of the DOC data. That is, growing hydrilla released organic molecules at both extremes of the size distribution where the THMFP was highest. It was interesting to note that the THM-precursors

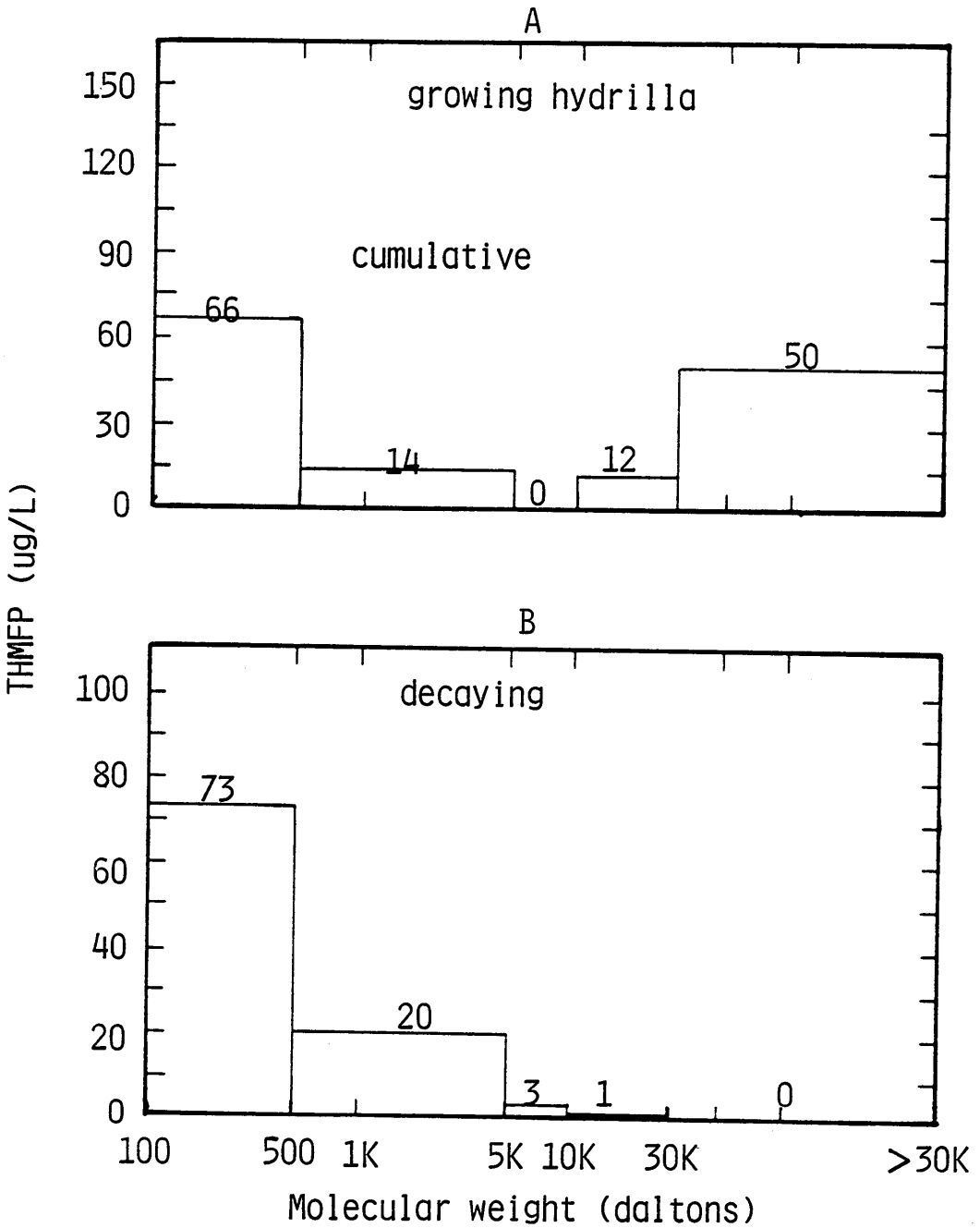


Figure 12. Size distribution of THM precursors released by growing hydrilla (top) and by decaying hydrilla (bottom).

concentrations in the 500- 5K and the 10K-30K dalton size fractions were low. Because there was no measurable organic carbon in these size ranges, it might be expected that no THMs would have formed upon chlorinaton. This discrepancy was most likely a result of error involved in either the ultrafiltration process or the analysis of THMs or DOCs.

Figure 12B illustrates the size distribution of the THM-precursors released by decaying hydrilla. Again, a difference was seen between the size distributions of the THM-precursor pool and the parent organic pool. Most of the THM precursors (93 percent) were in the two smaller size fractions, most being in the smallest fraction (100-500 dalton). The 30K and greater size fraction did not contain any THM-precursors, which was suprising because this fraction contained more of the DOC pool than any other.

Figure 13 compares the size distribution of ECP derived THM-precursors to that of the THM-precursors produced by the decay of hydrilla. The precursors released by hydrilla as it grew (ECP) were larger than those from the decaying hydrilla. Molecules from both sources (ECP and decay) in the small and the large size ranges accounted for more of the THM-precursors than those of intermediate size.

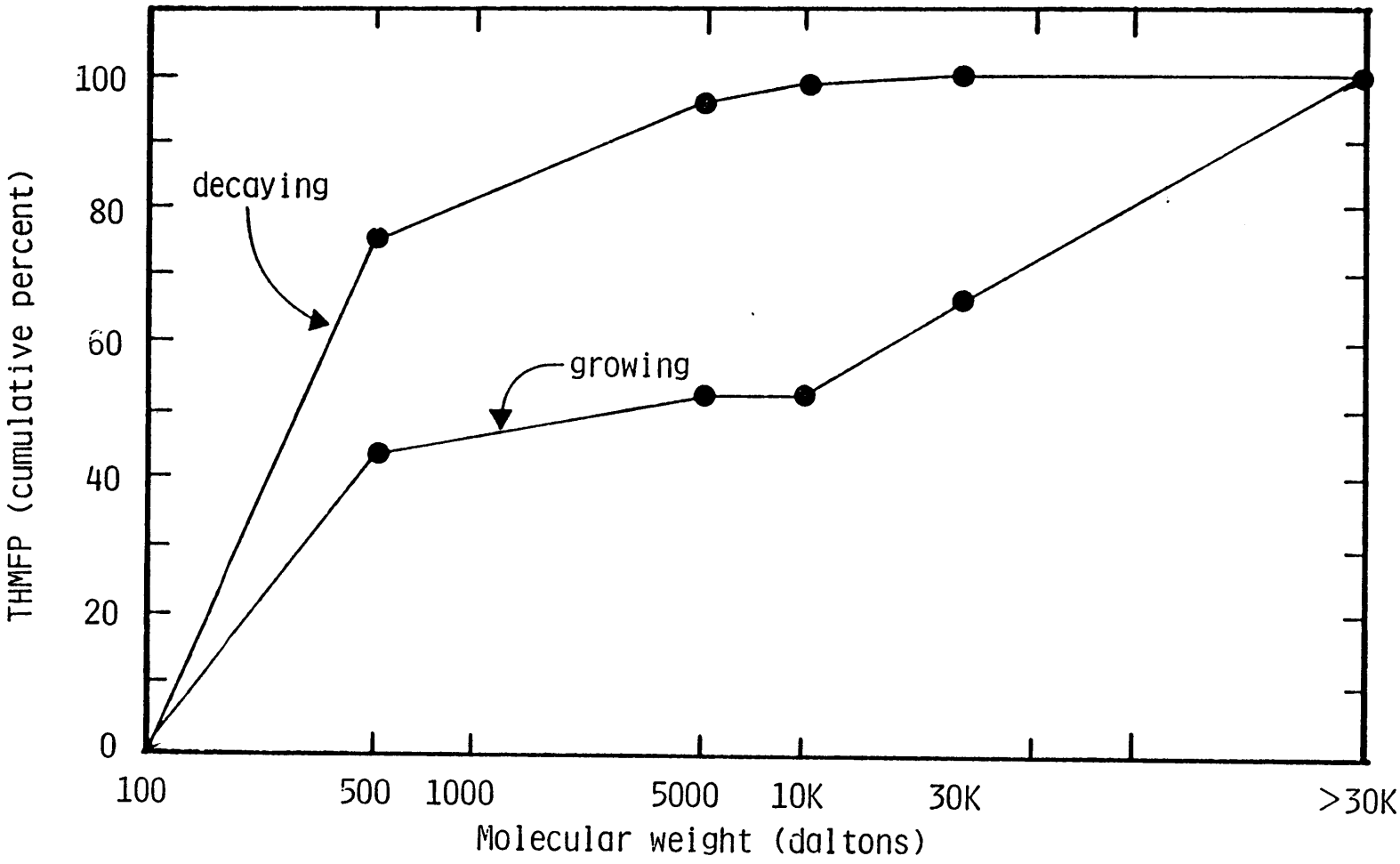


Figure 13. A comparison of cumulative THMFP distribution from organics released by growing hydrilla to that released by decaying hydrilla.

Yield at each size fraction

Interpretation of Yield Graphs

Yield is a measure of the capacity of DOC from a particular source to form THMs when chlorinated and, in this report, is expressed as ug THMFP per mg DOC (ug/mg). The cumulative yields in Figures 14 and 15 should not be confused with the yields of the bulk solutions because the cumulative yields are a meaningless quantities. The slopes of the lines in these graphs, however, give a relative comparison of the yields in each size catagory.

Molecular Weight Distribution of Yields

Figure 14A illustrates the yield of the various size ranges in the organic pool produced by growing hydrilla. As seen, the smaller organic molecules, which contributed a big percentage of the total organic pool, contained relatively few precursors resulting in a low yield for these low-molecular-weight molecules. Conversely, the THM yield from the pool of larger organic compounds (greater than 30 Kd) was quite high.

Figure 14B shows the THM yield from the various size organic substances released by and by decaying hydrilla. The pattern was quite different than that observed for the growing hydrilla. In this case, the smallest size fraction (100-500 daltons) contained the highest proportion of

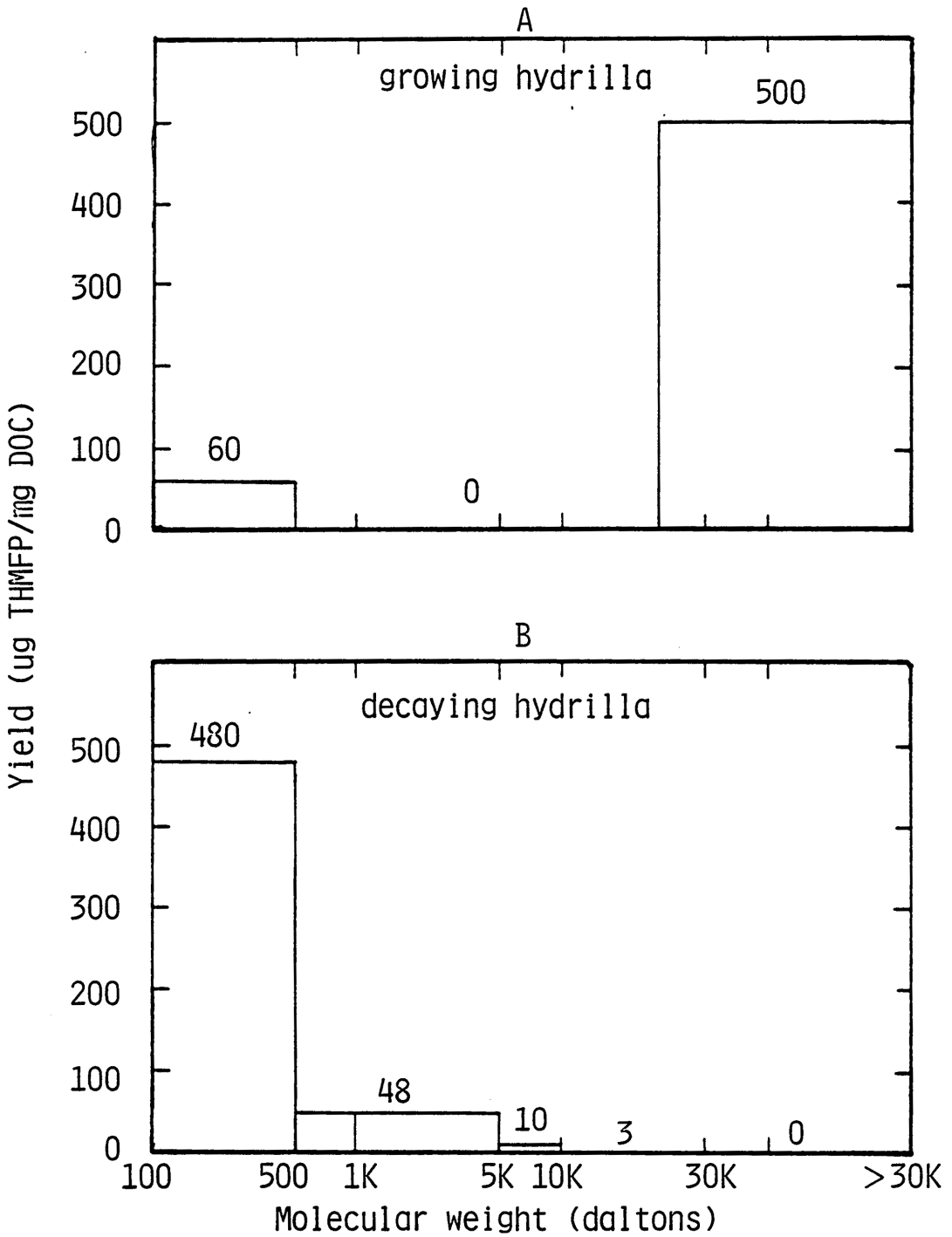


Figure 14. THM yield from the various size organic substances released by growing hydrilla (top) and decaying hydrilla (bottom).

THM-precursors. In summary, as the size of the organic molecules increased, the ability to form THM-precursors decreased.

The cumulative distribution of hydrilla-produced ECP is compared to that of hydrilla decay products humic acids in Figure 15. The actual data, rather than percentages, are presented in this figure. The distribution of the yield among the size ranges from these two sources was quite different. The distribution of the yields from the growing hydrilla was quite low, when compared to that of the decaying hydrilla until a size of 30K daltons, then increased rapidly. The organic molecules larger than 30K daltons contained enough precursors to give hydrilla ECP the same overall yield that resulted from decayed hydrilla.

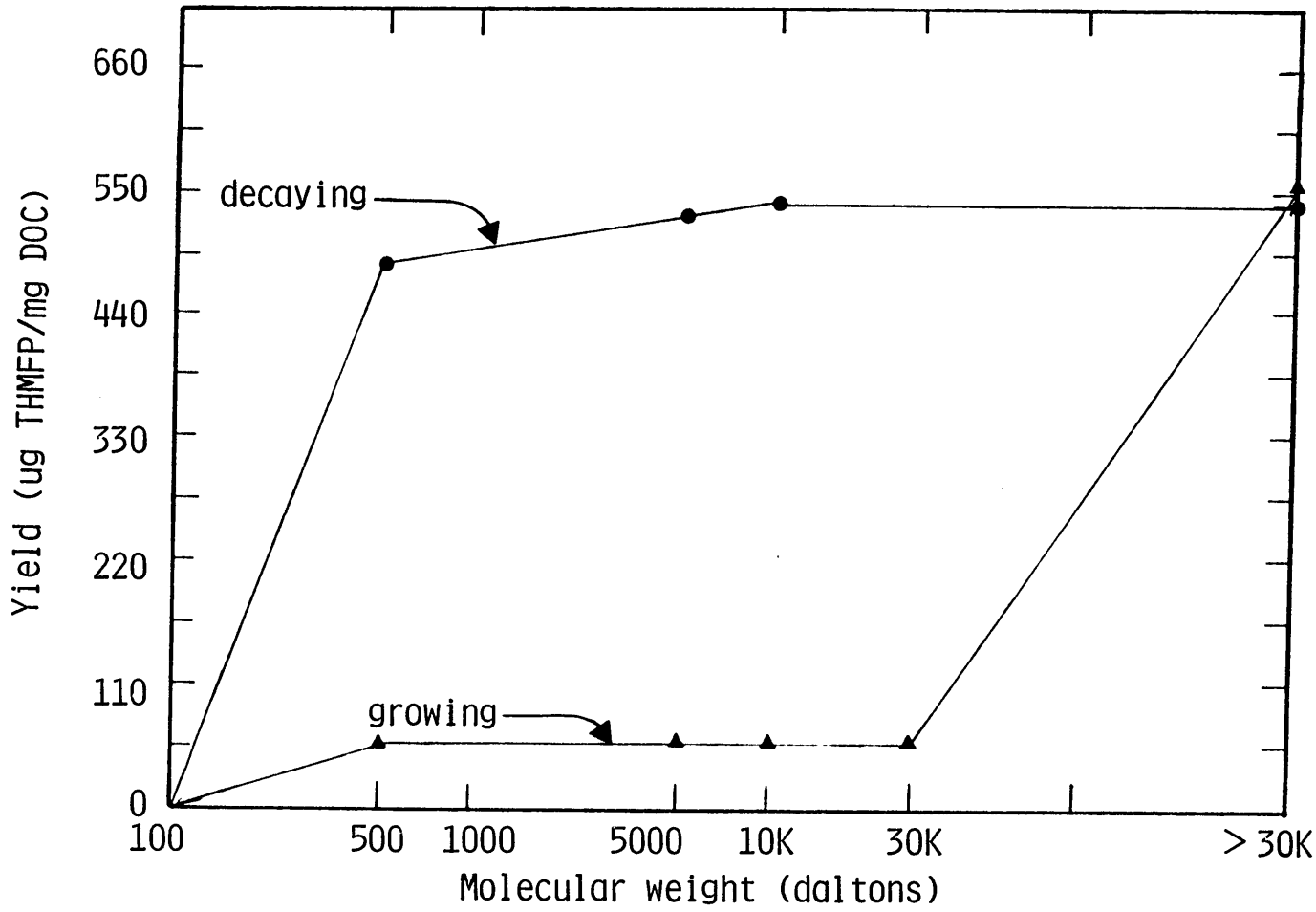


Figure 15. A comparison of cumulative yield between organics released by growing hydrilla and decaying hydrilla.

CHAPTER V

DISCUSSION

Effect Of Light On THM-Precursor Production

The ability of light to promote a diurnal pattern of THM-precursor production by hydrilla (as evidenced by algae) was not demonstrated in this study. Hoehn et al. (36) demonstrated that algae exhibited a definite diurnal pattern of THM-precursor production associated with the presence of extracellular product (ECP), which was produced as a by-product of photosynthesis. In other words, during hours of peak photosynthesis, ECP was present, resulting in a higher THMFP than existed during the night, probably because the ECP were metabolized by bacteria. No diurnal pattern of this sort was evident in hydrilla cultures (Figure 7). There were three possible reasons for this phenomenon. First, the growth rate of the hydrilla could have been so slow that ECP did not accumulate in significant quantities during the day. (The slow growth rate was evidenced by a constant pH which, would be expected to increase during periods of photosynthetic activity.) Second, ECP may have been produced in sufficient quantities to increase the THMFP of the medium, but the biodegradable fraction produced by hydrilla may have been so small that no reduction in THMFP was seen during the

dark hours (i.e. the substrate concentration was too small to support a bacterial population.) Third, the rates of THM-precursor production (ECP) and bacterial decomposition may have been equal.

Effect Of Sediment And Periphyton On THM-Precursor Production

Figure 7 indicated that sediment played a role in increasing the THM-precusor production by hydrilla. As mentioned, this was caused by more than just the leaching of organic compounds from the sediment into the water because sediment-related precursors in a control container (containing only nutrient mix and sediment) were subtracted before the final THMFP was calculated. During the course of the experiment, an algal bloom occurred in the two vessels containing sediment thus terminating the experiment. Most likely, the increase in THMFP was a result of periphyton that originated in sediment from the aquaria and colonized the surfaces of the hydrilla, not from organic compounds being released from the sediment itself. Of course, if hydrilla does serve as an attachment site for algae that produce THM-precursors, it is moot whether the hydrilla themselves were producing the precursors. The impact on the reservoir would be the same.

THM-Precursor Production During the Four Experiments

Table 8 compares the THMFP data derived from the four experiments. Because the length of the study periods varied, THM-precursor production was normalized by expressing it as a rate. The rate of THM-precursor production was fastest for Experiment IV. The turbulent nature of this microcosm, which may be similar to that created by wave action in a natural setting, caused THM-precursors to be released at a rate five-ten times faster that in the other Experiments. It was also interesting to note that the release rate of ECP-derived THM-precursors in quiescent microcosms (Experiments I and II) was similar to that of decay-derived THM-precursors (Experiment III).

Effect Of Hydrilla On The THMFP Of Occoquan Water

The approximate increase in THMFP of the reservoir was estimated using 1) literature data on plant density per surface area of lake bottom; 2) the fraction of lake bottom that is suitable for colonization by hydrilla; and 3) the volume of water contained in the reservoir. These data were presented earlier in the Literature Review.

Determination of THM-Precursor Increase Due to Hydrilla

Two terms, 1) the fraction of the reservoir's volume that would be available to dilute the hydrilla-derived THM-precursors, and 2) the quantity of THM-precursors re-

Table 8.
Contributions of THM-Precursors from all experiments.

Precursor Source	THMFP (ug/g-dry-day)
Experiment I (sediment)	9[1]
Experiment I (no sediment)	6[1]
Experiment II (light)	16[2]
Experiment II (dark)	11[2]
Experiment III (decaying)	15[1]
Experiment IV (turbulent)	80[1]

[1] last data taken
[2] average values

leased by a unit mass of hydrilla (ug/g-dry), had to be determined to predict impact of hydrilla on the THM-precursor levels present in the reservoir. It was assumed that the precursors will be distributed evenly throughout the water column. During stratification, higher concentrations of THM-precursor may be localized in the waters overlaying the hydrilla beds. During overturn, however, it is expected that the precursors will be well mixed with the reservoir's entire volume of water.

Second, the contribution of THM-precursors from a unit mass of hydrilla had to be derived. By themselves, each of the experiments (I-IV) was designed to measure a single source of THM-precursors (by decay or by ECP production), not the total amount of THM-precursors that would be released by a unit mass of plant. To arrive at an overall measure of the total THM-precursor production by hydrilla, data from Experiments I and III (see Methods, pages 28-30 and Table 8) were summed to yield a value of 500 ug/g-dry. Table 8 shows, however, that the hydrilla from Experiment IV released more that this (1,000 ug/g-dry). As is explained in more detail in the following section, the hydrilla in Experiment IV probably released THM-precursors to the supporting media as a result of both the release of ECP and decay products. To simulate worst case conditions, the greater value of 1,000 ug/L from Experiment IV is used in subsequent calulations.

Calculation of the increase in THMFP

The following formula was used to find the increase in THMFP that would result from an infestation of hydrilla in the Occoquan Reservoir:

$$TI = ADT/V \qquad [1]$$

where:

TI = THMFP increase (mg/m^3)

A = colonized area of lake bottom ($2.4 \times 10^6 m^2$)

D = plant density (400 g-dry/m^2)

V = reservoir volume ($42.4 \times 10^6 m^3$)

T = THM-precursors from hydrilla (1.0mg/g-dry)

Thus:

TI = 23 ug/L.

Because the THMFP of the Occoquan Reservoir normally is in the range of 1,000 ug/L (20) hydrilla would not significantly increase it so long as the mass of THM-precursor produced by hydrilla is equal to or less than the mass of THM-precursors released by the outlet.

Yields

The yield of THMs from the dissolved organic carbon (DOC) concentration in a water sample provided a measure of the capacity of the various DOC pools to form THMs when chlorinated. Figures 16 and 17 showed that there was no well-defined relationship between the THMFP and the DOC derived from the hydrilla culture media in either Experiment I

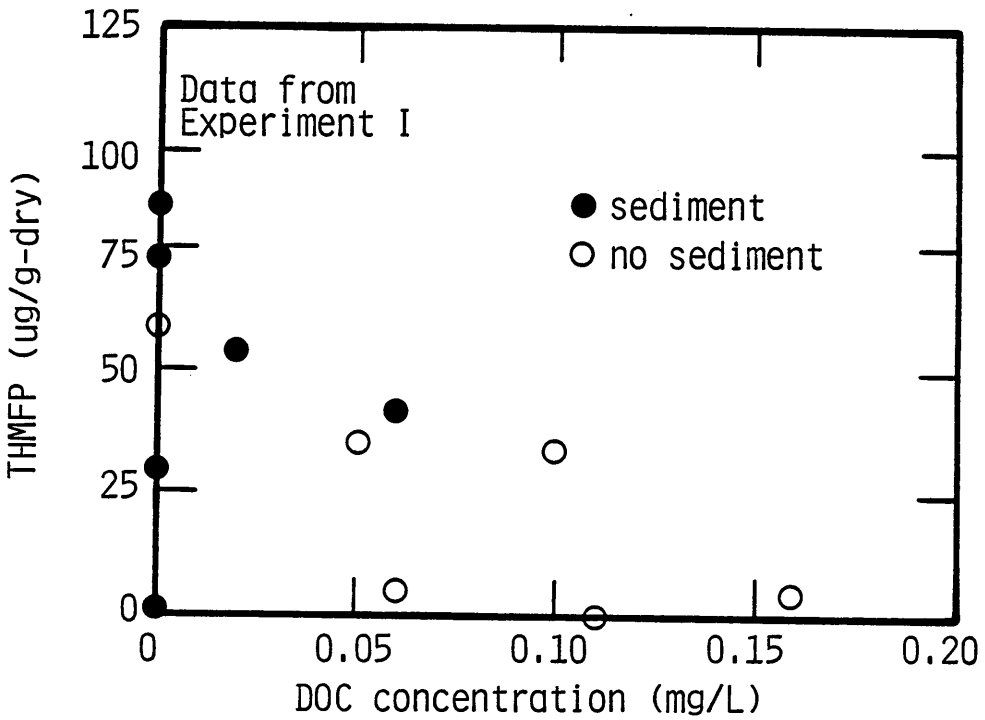


Figure 16. Trihalomethane formation potential (THMFP) vs. dissolved organic carbon (DOC) for Experiment II.

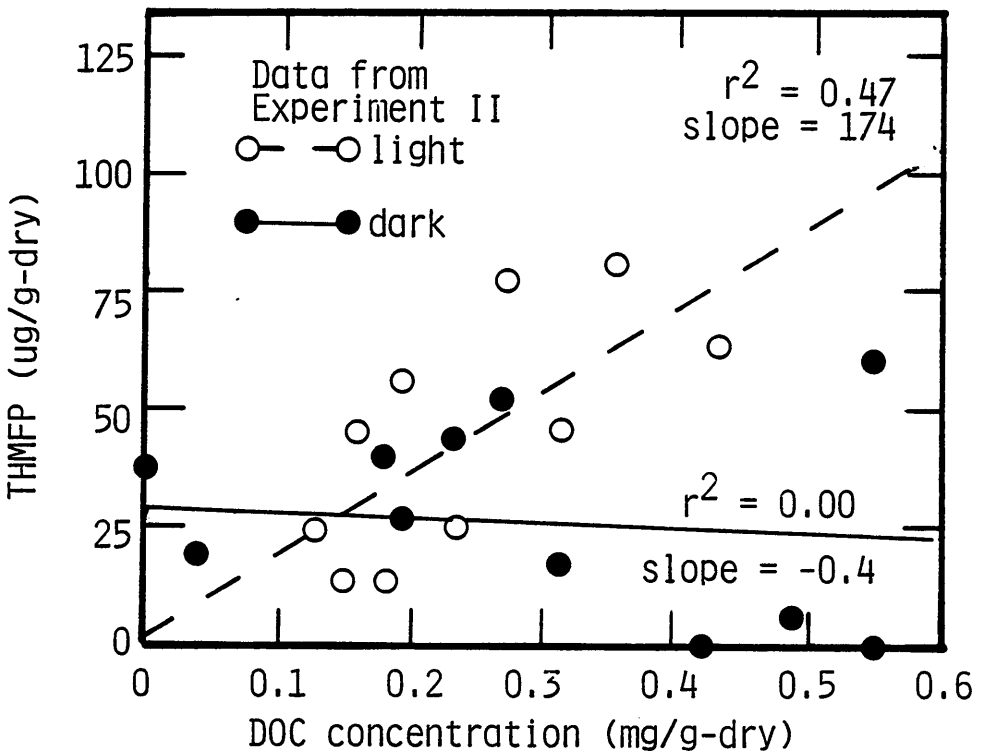


Figure 17. Trihalomethane formation potential (THMFP) vs. dissolved organic carbon (DOC) for Experiment II.

or Experiment II (see Methods, pages 28-29). In contrast, Figures 18A and 18B show that a better defined relationship exists between the DOC and THMFP of the media used in Experiments III and IV (see Methods, page 29-30). An average yield was calculated from data derived from Experiments I and II, and the slopes of the lines from Figures 18A and 18B were used to derive a yield from data collected during Experiments III and IV. In Table 9, these data are summarized and contrasted with literature values.

The yields from the four experiments were quite variable, ranging from a high of 10,000 ug/mg (100 percent yield of THM from the available DOC) in the media containing from hydrilla in sediment in Experiment I, to a low of 30 from the DOC released by decaying hydrilla in Experiment IV. The extreme THM yields from the DOC in the media in Experiment I (especially for the sediment group) were caused by the low DOC values (often zero) that resulted when the DOC of the control group was subtracted from that of the experimental group. The ranges presented for Experiment I were calculated only for those cases where a measurable difference in DOC existed between the experimental and control groups (Table 6).

The THM yields from the media used in Experiment II were comparable in both range and magnitude to those reported for algal ECP and biomass (8).

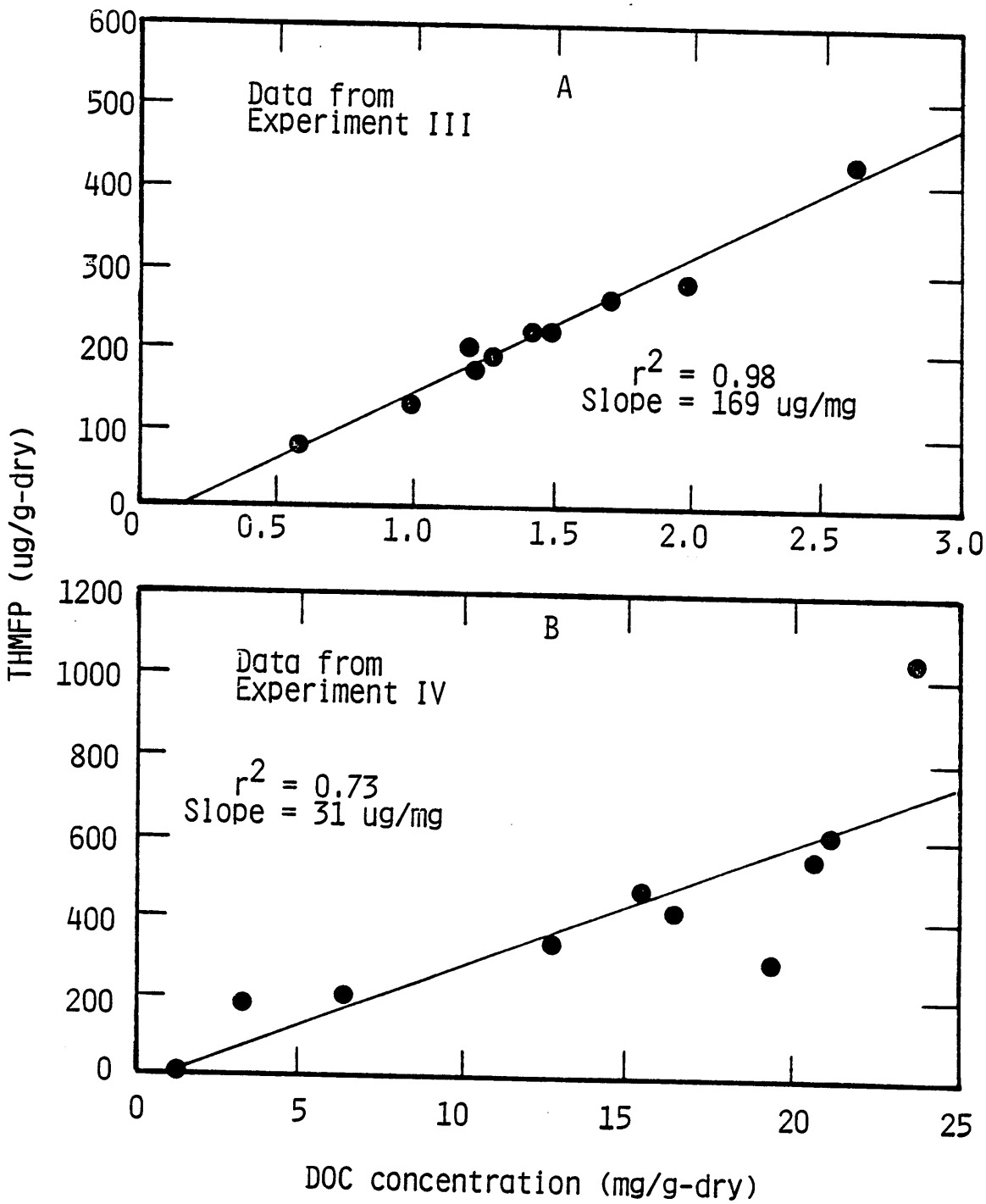


Figure 18. Relationship between trihalomethane formation potential (THMFP) and dissolved organic carbon (DOC) concentration derived from hydrilla from Experiment III (A) and Experiment IV (B).

Table 9.
THM yields from the DOC of the four experiments in this study compared to those found in the literature.

Precursor Source	Yield (ug/mg)
Experiment I (sediment)	700-10,000
Experiment I (no sediment)	0-700
Experiment II (light)	28-294
Experiment II (dark)	0-455
Experiment III (decaying)	170
Experiment IV (turbulent)	30
Occoquan Reservoir (23)	130
Fulvic Acids (37)	29-51
Humic Acids (37)	61-77
Algal ECP (8)	4-500
Algal biomass (8)	20-400

The yield of the hydrilla decay products (Experimment III) is greater that reported for humic substances but similar to what is reported for Occoquan Reservoir water.

The yield of THM from the DOC in the media of Experiment IV was the lowest encountered in this study. Thus, even though turbulence caused hydrilla to release THM-precursors at a high rate (Table 8), it caused hydrilla to release DOC at even a greater rate when compared to the other experiments in this study.

The THM yields from the DOC released in a turbulent microcosm (Experiment IV) revealed a further difference in THM-precursor production produced by water movement. The relationship between THMFP and DOC from this experiment was more consistant than that observed in either Experiment I or Experiment II. Thus, turbulence caused hydrilla to release DOC at a constant rate, and with a consistant yield.

Molecular Characterization Of Organics Released By Hydrilla

Hydrilla ECP

The organic compounds released by growing hydrilla were quite different than the organic compounds from other sources analyzed in this study. Most likely, they were released as a by-product of photosynthesis. Unlike most naturally occurring organic pools, which often contain organic compounds of many sizes, hydrilla ECP falls into only two size divi-

sions, less than 500 and greater than 30,000 daltons. In addition to being different in size, the two size ranges contained different proportions of precursors as evidenced by a difference in yields (Figure 14A). The smaller compounds had a relatively low yield, whereas the larger ones had a high yield. This was somewhat unexpected because the smaller organic compounds would be expected to have more "sites of attack" for chlorine per carbon atom than would the large organic molecules (which often have many carbon atoms sequestered within the interior of the molecule).

Organics Released by Decaying Hydrilla

The MW distribution of the organic pool released by decaying hydrilla was not as distinctive as that from hydrilla ECP. The similarity of the MW distribution derived from hydrilla decay products to those of naturally occuring organic compounds (Figure 11 compared to Figure 4) was not unexpected, because most organic compounds in the aquatic environment are the result of bacterial decay (as promoted in Experiment III).

The MW distribution of these organic compounds released by decaying hydrilla was unique in one respect, namely that a significant quantity of DOC appeared in each of the size ranges. In contrast, the size distributions of the organic compounds from literature sources (Figure 4) were predomi-

nated by one or two size divisions, as indicated by one or two regions of steep slope.

The MW distribution of THM-precursors released from decaying hydrilla (Figures 12 and 13) was not as well distributed as that of the corresponding DOC pool. In addition, the size distribution of THM-precursors differed significantly from that of THM-precursors from commercial humic acids. Most notably, the larger organic compounds released from decaying hydrilla were low yield THM-precursors, resulting in a much different MW THM-precursor distribution than was exhibited by humic acids. The absence of THM-precursors with MW greater than 5K daltons further differentiates the organic compounds produced by decaying hydrilla both from humic acids and from other naturally occurring organic pools (Figure 4). The absence of THM-precursors among the high-MW organic fraction resulted in low THM yields (Figure 13) and may indicate that the higher MW organic compounds did not have the functional groups responsible for the THM reaction (e.g. hydroxyl groups in the meta position).

Effect On The Occoquan Reservoir

Hydrilla ECP

Figure 19 compares the molecular-weight distributions of organic compounds released by living and decaying hydrilla to those of algal ECP (20) and the Occoquan Reservoir (23).

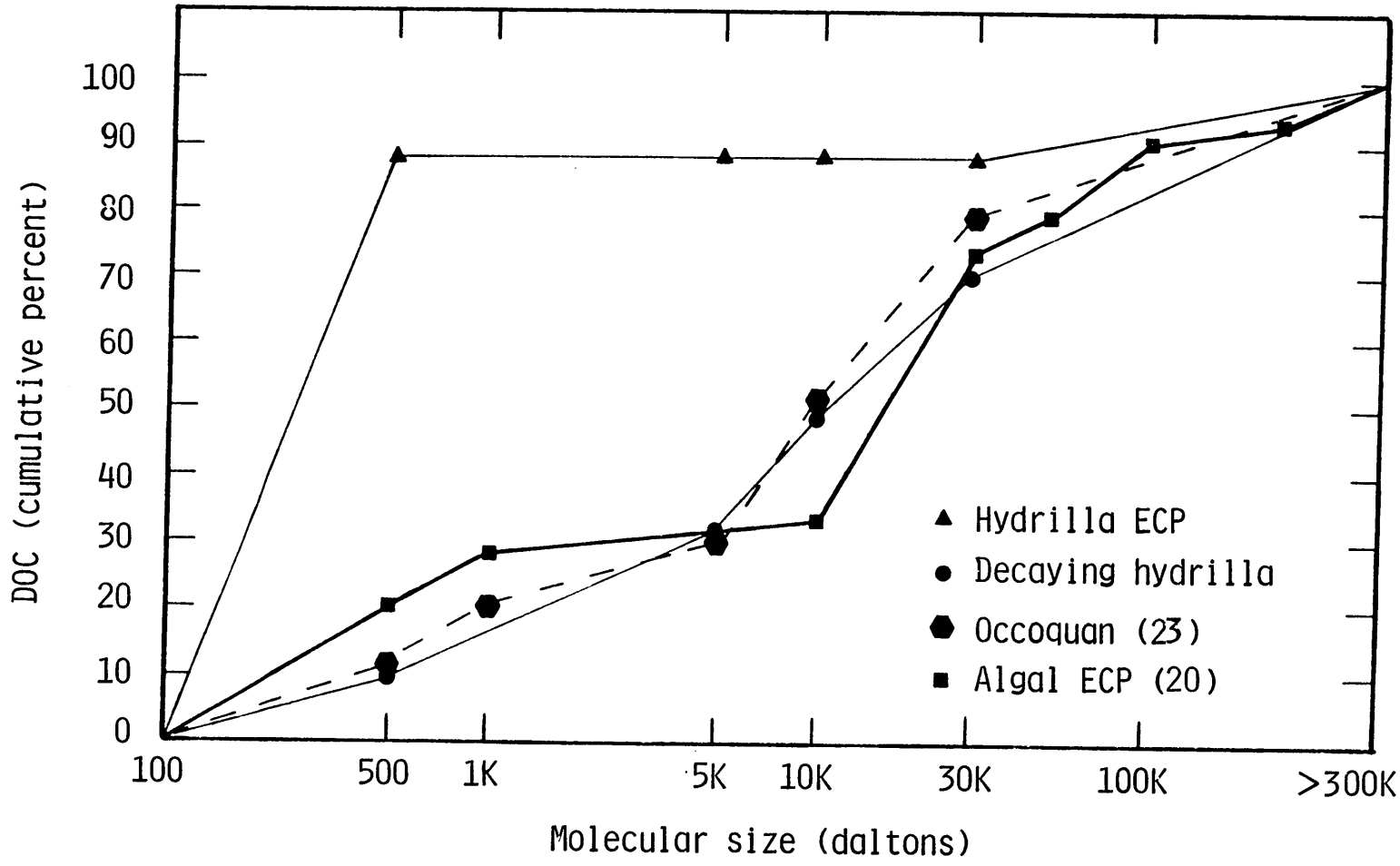


Figure 19. Molecular-weight distributions from hydrilla compared to those of algal ECP (20) and Occoquan Reservoir water (23).

As can be seen, the ECP released by hydrilla were significantly smaller than those released by algae, which suggests that this organic pool originated from hydrilla, not the periphyton that was thought to be associated with it in Experiment I. In addition, this figure showed that should the contribution of organic carbon from hydrilla ECP to the total organic pool in the Occoquan become significant, the molecular weight distribution of the Occoquan's organic pool would be shifted towards lower weight organic compounds. The likelihood of this was thought to be low for two reasons: First, hydrilla released low levels of organic carbon and, second, smaller organic molecules are more short-lived in the environment due both to polymerization and to bacterial decay (24).

The major effect of hydrilla ECP may be on the THMFP of the Reservoir. Although the small organic compounds had a low yield, they contributed more THM-precursors to the small size ranges than was found in the Occoquan (Figure 20). These organic compounds, by virtue of their small size, however, may be more amenable to decay. However, should these small precursors escape decay they would be difficult to remove in the coagulation step in the water treatment plant and would effect the THMFP of the finished water.

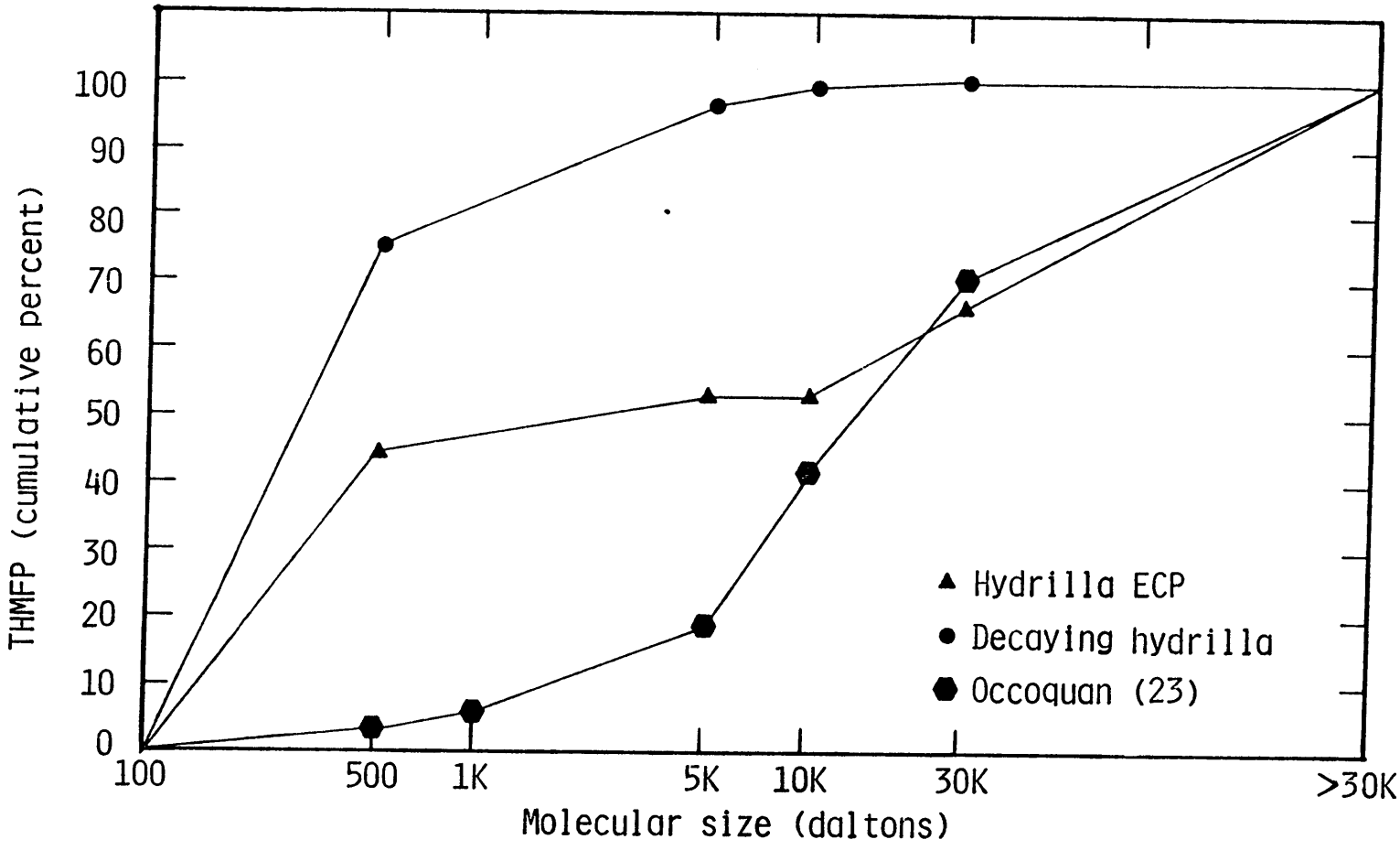


Figure 20. Molecular-weight distribution of THM-precursors from hydrilla compared to that of Occoquan Reservoir waters (23).

Decaying hydrilla

Figure 19 shows that the MW distribution of organic compounds released by decaying hydrilla was similar to that of the organic compounds in Occoquan Reservoir water. Consequently, the contribution of organic compounds by decaying hydrilla would not be likely to alter the existing MW distribution of the organic compounds in the reservoir, no matter how much of a decaying mass accumulates. Figure 20 however, shows that the distribution of THM-precursors from decaying hydrilla was much smaller than the ones already existing in the Occoquan. Thus, an accumulation of organic compounds from decaying hydrilla may be expected to increase the amount of THM-precursors with MW less than 5K daltons in the Occoquan Reservoir. Because low MW organic compounds are more difficult to remove by coagulation, the THMFP of the finished waters may be increased should decaying hydrilla accumulate to an appreciable extent. This statement is conditional because of the possibility that polymerization or bacterial decay ofthese organic substances may either produce or eliminate THM-precursors.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Hydrilla has become a nuisance weed in many of Florida's surface waters. This plant is now moving north and has been found in the Potomac River. There were two concerns regarding the possible proliferation of hydrilla in Virginia. The first, more traditional, concern is over the problems associated with the choking of valuable recreational areas and the second, more contemporary concern is over the possible production of THM-precursors by hydrilla. The Occoquan Reservoir, a water supply to over 600,000 people, already contains significant levels of THM-precursors. Thus, the addition of THM-precursors by hydrilla is a serious concern. The conclusions summarized below address these concerns.

- The nutrient levels in the water and the sediment of the Occoquan will support hydrilla should it establish itself in the basin.

- Once established, hydrilla will contribute to both the DOC and the THM pool. The THM-precursor pool is composed of three fractions as follows: 1) Extracellular products (ECP) from hydrilla; 2) ECP from periphytonous growth

attached to hydrilla; and 3) decay products from hydrilla.

- Calculations based both on estimates of the maximum reservoir area that can be colonized by hydrilla and on laboratory-derived data regarding THM-precursor production by hydrilla reveal that a prolific infestation of hydrilla in the Occoquan Reservoir would elevate current THMFPs by about 24 ug/L, which is about 2.5 percent of current levels. Over a period of years, the accumulation of decaying hydrilla might elevate the THM-precursor level even more, but likely never to levels that would present serious water-treatment difficulties unless the maximum contaminant level for THMs is reduced considerably. If that occurs, the organic matter already present would pose a more serious problem to the Fairfax County Water Authority than would the THM-precursors contributed by hydrilla.

- Hydrilla ECP are smaller than many other naturally occurring organic substances in the aquatic environment and have a yield in the range of 0-500 ug THM/mg DOC.

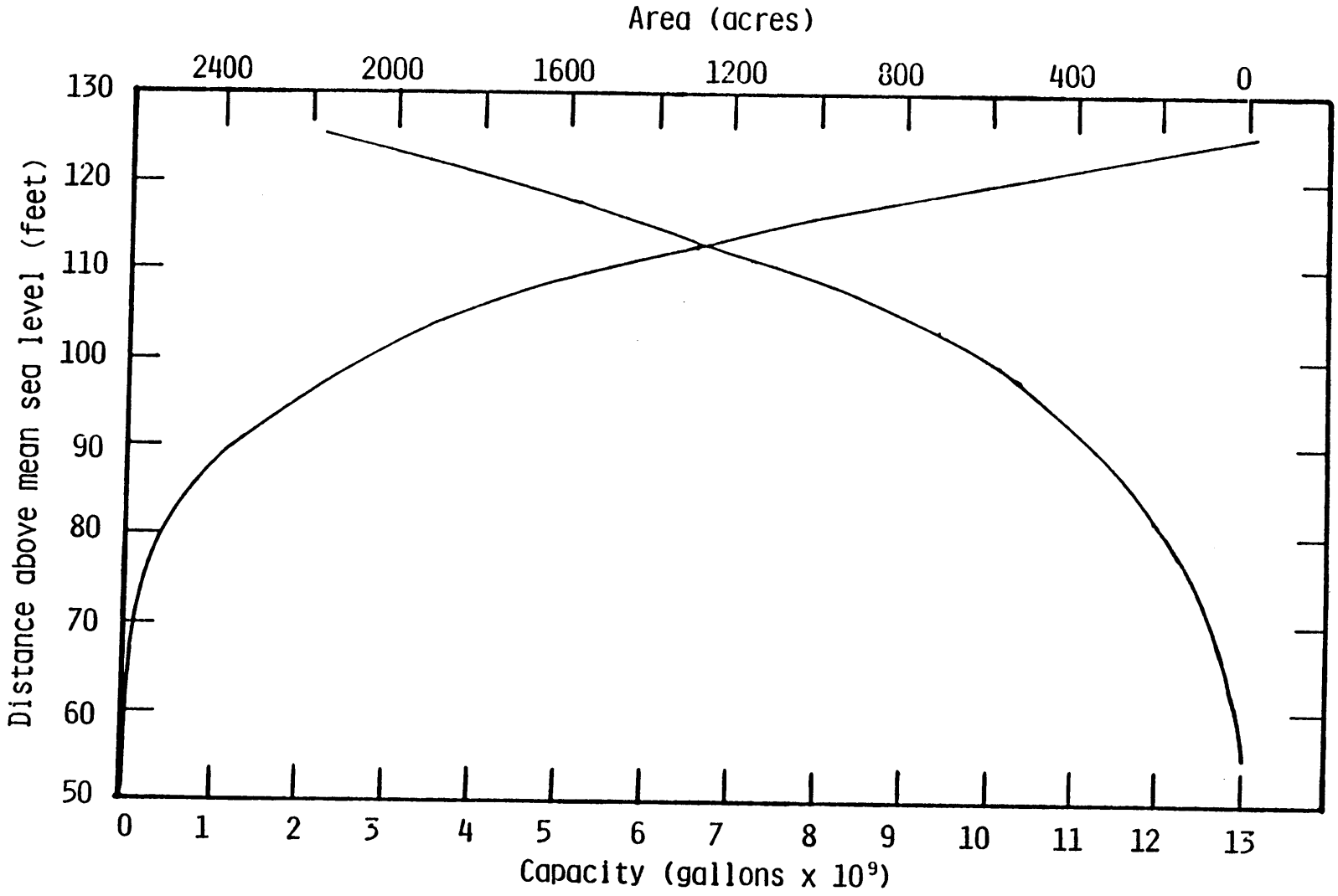
- Hydrilla decay products have a molecular-weight distribution that is similar to many other naturally occurring

organic compounds in the aquatic environment and have a yield of about 170 ug THM/mg DOC.

APPENDIX A

HIPSOGRAPIC CURVE FOR THE OCCOQUAN RESERVOIR

Appendix A. Hipsographic curve for the Occoquan Reservoir, 24 January, 1977 from Thomas J. Grizzard Ph.D. (unpublished data).



APPENDIX B

NUTRIENT SOLUTION USED IN HYDRILLA EXPERIMENTS

Nutrient solution used in hydrilla experiments.

Macrosalt	Concentration (mg/L)	Element	Concentration (mg/L)
$CaCl_2$	88.80	Ca	32.10
K_2HPO_4	69.60	K	15.60
$NaNO_3$	680.00	N	112.00
$MgSO_4 \cdot 7H_2O$	96.30	Mg	18.60
$MgCl_2 \cdot 6H_2O$	76.10	S	12.50
Na_2HPO_4	56.80	P	90.10
Microsalt			
H_3BO_3	9.10	B	1.6
$MnCl_2 \cdot 2H_2O$	3.50	Mn	0.97
$CuCl_2 \cdot 2H_2O$	0.0004	Cl	87.20
		Cu	0.0015
$ZnCl_2$	1.80	Zn	0.86
$FeCl_3$	2.60	Fe	0.89
$CoCl_2$	0.04	Co	0.018

APPENDIX C

WET-WEIGHT AND DRY-WEIGHT COMPARISONS

Wet-weight and dry-weight comparisons.

Wet-weight (grams)	Dry-weight (grams)	percent dry
2.516	0.188	7.49
1.610	0.116	7.23
3.047	0.147	4.83
1.584	0.085	5.38
0.988	0.060	6.10
1.160	0.066	5.69
0.894	0.047	5.29
0.976	0.052	5.08
0.540	0.037	6.82
1.619	0.090	5.66
0.508	0.031	6.10
	mean value =	5.96

APPENDIX D

DATA FROM EXPERIMENT 3

Experiment 3: Hydrilla (0.598 g-dry) allowed to decay in 2.5 Liters of organic free water.

Day	volume (ml)	TOC (mg/L)	TTHMFP (mg/L)	TOC (mg/g-dry)	TTHMFP (ug/g-dry)
0.00	50	1.19	24	1.98	100
1.00	50	0.79	48	1.27	197
2.30	50	1.03	26	1.72	104
2.70	50	0.74	43	1.23	169
3.20	50	0.35	20	0.59	77
5.80	50	0.72	45	1.20	190
8.80	50	0.59	34	0.98	125
11.00	50	0.89	63	1.48	225
12.20	50	0.86	63	1.43	220
15.20	50	1.03	77	1.72	263
18.00	50	1.20	75	2.00	280
25.90	50	1.57	117	2.62	430

APPENDIX E

DATA FROM EXPERIMENT 4

Experiment 4: TOC and TTHMFP from 1.630 g (dry) hydrilla in a stirred microcosm.

Day	Time	TOC (mg/L) exp	TOC (mg/L) cont	THMFP (ug/L) exp	THMFP (ug/L) cont	TOC mg/g-dry	THMFP ug/g-dry
0.0	7.30	0.75	0.35	9	10	1.20	0
1.7	17.30	1.35	0.36	67	11	3.04	175
4.1	7.30	2.50	0.43	79	13	6.35	203
5.7	15.00	4.41	0.35	124	16	12.45	332
7.0	9.30	5.67	0.32	147	11	16.41	417
8.7	7.40	5.49	0.47	200	46	15.40	472
9.7	7.00	7.22	0.49	203	23	20.60	552
10.2	12.00	6.97	0.68	131	31	19.29	307
10.9	11.00	7.64	0.70	213	20	21.30	592
12.8	15.00	8.75	1.01	376	38	23.70	1037

Note: exp = experimental (stirred microcosm containing hydrilla)
cont = control (nutrient solution only, stirred continously)

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