EFFECTS OF ORGANIC FRACTIONS FROM SECONDARY EFFLUENT ON SELENASTRUM CAPICORNUTUM (KUTZ)

BY

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Eutrophication of lakes is associated with an increase in primary production, depletion of oxygen, production of odors, and in extreme cases excessive algal blooms.

The algal crop in a body of water is dependent on many variables such as type and concentration of nutrients, algal genera, climate, sunlight, and many other factors, depending on the physical and environmental conditions affecting the water body.[1] Domestic as well as industrial wastes are generally the main contributors of carbon, nitrogen, and phosphorus in natural water bodies. These elements and their compounds have often been implicated as the prime chemical causative agents to the eutrophication phenomenon.[2]

Algae use carbon dioxide from the atmosphere as well as from carbonate-bicarbonate alkalinity equilibria in waters. In addition, some of the blue-green algae are known to fix nitrogen from the atmosphere.[3] Phosphorus is a major element which does not gain access to natural waters through the gaseous phase and of all the factors affecting a lake's stability, it is considered to be the most easily controllable parameter. Much controversy regarding the likely impact of phosphorus control and the best methodology to achieve it, has occurred within the scientific community in recent years.[4]

To date, however, much less attention has been focused on the effect of dissolved organic fractions in secondary treated effluents on algal stimulation. It has been shown that concentrated secondary treated effluent and certain fractions thereof stimulated *Selenastrum capricornutum* and *Anabaena flos-aquae*.[5, 6] The effect of secondary and tertiary treated wastewater effluents on the waters of the Shagawa Lake (MN) and Burntside River (MN) was evaluated with *S. capricornutum* as the test organism.[7] The secondary treated effluent with a final concentration of 10 percent in the medium elicited considerable stimulation of this alga but the tertiary treated effluent had no effect. Middlebrooks *et al.*[8] showed that raw wastewater and effluents from primary, secondary, and tertiary treated wastewater elicited stimulation for *S. gracile* up to 10 percent final concentration in the medium whereas the final concentration of secondary and tertiary treated effluents in the medium from 10 to 50 percent were inhibitory. Pipes[9] concluded that several dissolved organic compounds in wastewater accelerated the growth of *Chlorella sp.* and served as an additional carbon source for this alga. He also showed that some fractions indicated stimulation even when carbon dioxide was not limiting. Bender *et al.*[10] felt that there were organic substances in secondary wastewater effluent which chelated the trace metals and thus stimulated the algal growth. Prakash and Rashid [11] concluded that the stimulatory effect of humic substances on algae, for the most part, was independent of nutrient concentration and that humic substances acted as specific sensitizing agents which enhanced the permeability of the cell, thus allowing increased uptake of the nutrients.

In this study an attempt has been made to determine if dissolved organic compounds of different molecular weights existing in secondary treated domestic wastewater effluent stimulate algal growth. While some work has been done, it remains to be seen if these findings are more universally observable. It has been shown in the current study that the nutrients such as phosphorus, nitrogen, and inorganic carbon in wastewater effluents did not contribute to stimulation of the test algal species, whereas the organic components did show stimulatory effects.

METHODS OF PROCEDURE

Approximately 120 l of secondary treated wastewater effluent was collected from each of two treatment plants treating domestic wastewater. The Lake George (N. Y.) wastewater treatment plant employs a trickling filter system of treatment while Clifton Knolls (N. Y.) is a contact stabilization plant. The effluents were immediately membrane filtered (0.45 nm pore size) and subsequently concentrated via the freeze-drying technique. These effluents were concentrated 103 and 112 times respectively.

Sephadex Gels G-10 (fractionation range 0 to 700 mol wt), G-15 (range 0 to 1 500 mol wt), and G-25 (range 1 000 to 5 000 mol wt) were employed for chromatographic separations of the concentrated effluents. Apparent molecular weights (AMW) were assigned to these fractions from the standard curves developed for each Sephadex column.[12] From 7 to 10 ml of each concentrated effluent was applied separately to a Sephadex G-10 column, and five independent fractions were obtained in each case. This procedure was repeated several times until a large enough quantity of each fraction was collected. These fractions, in turn, were concentrated via freeze-drying for further fractionation. The concentrated frontal fraction from the Sephadex G-10 column (that is, AMW >700) was applied to a Sephadex G-15 column, and two fractions were obtained in the case of each wastewater effluent. A large enough quantity of each fraction was collected by repeated separations and then concentrated. The concentrated frontal fraction from the Sephadex G-15 column (AMW > 1 500) of the two effluents was separately applied to a Sephadex G-25 column and only one fraction was obtained in each case. A sufficient quantity of the fraction was collected by repeated sequential fractionation and subsequent concentration. A carbonaceous analyzer was used to measure organic carbon in the wastewater effluents, the concentrated effluents, and the various chromatographed fractions.

Depending upon the organic carbon content of each fraction, its percent concentration in the wastewater effluent was determined. Tables I and II contain the percent concentration of each fraction in the effluent, as well as its organic carbon concentration.

The effect of an effluent or an organic fraction on algal growth was determined by adding it to an algal growth medium and comparing the growth response with that of a control. The quantity of the concentrated effluent or the concentrated organic fraction to be added to 100 ml of the medium was calculated (see Tables I and II) to produce the same concentration of this component as in the plant effluent.

The bioassay procedure of the Joint Industry-Government Task Force on Eutrophication [13] (Provisional Algal Assay Procedure,

TABLE I. Percent of organic fractions in Lake George water pollution control facility effluent and their dosage for algal cultures.

Organic Fraction	Apparent Molecular Weight	Percent Organic Carbon in Unconcentrated Effluent (%)	Organic Carbon in Unconcentrated Effluent (mg/l)	Organic Carbon in Concentrated Fraction (mg/l)	Dosage of Fraction to Be Added to 100 ml Medium (ml)	Concentration of Supplemental P in the medium (mg/l*)	Concentration of Supplemental N in the medium (mg/l*)
Concentrated Effluent		100.0	8.66	645.0	1.34	0.064	2.29
G-10-I	>700	37.8	3.27	780.0	0.42	0.011	0.006
G-10-II	430	33.7	2.92	1 225.0	0.24	0.015	0.000 5
G-10-III	117	7.1	0.61	370.0	0.17	0.000 3	0
G-10-IV	Undefined	7.3	0.63	405.0	0.16	0.000 4	0.835
G-10-V	Undefined	6.7	0.58	202.5	0.29	0.004	1.670
G-15-I	>1 500	20.4	1.77	200.0	0.88	—	—
G-15-II	1 200	17.4	1.50	475.0	0.32	—	—
G-25-I	>5 000	20.4	1.77	385.0	0.46	—	—

* The concentration of P and N are in addition to those in the NAAM medium (Table III) and are supplemental to the medium as a result of the addition of the organic fraction.

TABLE II. Percent of organic fractions in Clifton Knolls water pollution control facility effluent and dosage for algal cultures.

Organic Fraction	Apparent Molecular Weight	Percent Organic Carbon in Unconcentrated Effluent (%)	Organic Carbon in Unconcentrated Effluent (mg/l)	Organic Carbon in Concentrated Fraction (mg/l)	Dosage of Fraction to Be Added to 100 ml Medium (ml)	Concentration of Supplemental P in the medium (mg/l*)	Concentration of Supplemental N in the medium (mg/l*)
Concentrated Effluent		100.0	11.14	930.0	1.20	1.050	0.199
G-10-I	>700	41.5	4.62	1 720.0	0.27	0.015	0.010
G-10-II	310	31.6	3.52	937.5	0.38	1.087	0.012
G-10-III	100	6.3	0.70	150.0	0.47	0.008	0.003
G-10-IV	Undefined	14.6	1.63	535.0	0.30	0.002	0.006
G-10-V	Undefined	1.6	0.18	47.5	0.38	0.001	0.002
G-15-I	>1 500	28.8	3.21	580.0	0.55	—	—
G-15-II	750	12.7	1.41	580.0	0.24	—	—
G-25-I	>5 000	28.8	3.21	345.0	0.93	—	—

* These concentrations of P and N are in addition to those in the NAAM medium (Table III) and are supplemental to the medium as a result of the addition of the organic fraction.

PAAP) was strictly followed. *S. capricornutum* (a green alga) was employed as the test alga and was obtained from the National Eutrophication Research Program, Pacific Northwest Laboratory, U. S. Environmental Protection Agency, Corvallis, Oreg. One hundred ml of the New Algal Assay Medium (NAAM)* was used in 500-ml flasks which were covered with foam plugs for efficient gas exchange. The constituents of the medium are given in Table III. The following environmental conditions were maintained: light intensity at the surface of the cultures, 400 ± 10 percent foot candles; oscillations per minute, 110; starting cell concentration, 10^3 cells/ml; and temperature in culture room 24° ± 1°C. The growth response of the controls as well as the cultures with organic fractions added to them were followed daily by cell number using the spectrophotometric technique,[14] with a spectrophotometer at 600 nm with a 5-cm light path. Three replicates were prepared in each case for the cultures with organic fractions as well as for the controls. The carbon-14 technique was also employed for a separate set of experiments and is described elsewhere.[14]

RESULTS AND DISCUSSION

The extent of stimulation or inhibition of growth of *S. capricornutum* resulting from the addition of various organic fractions was obtained by the calculation of maximum specific growth rates (μ_{max}) and maximum standing crops. The μ_{max} was calculated by the Environmental Protection Agency[15] method and the regression analysis. The maximum standing crop in a culture was assumed to have been achieved when the biomass increased less than 5 percent per day.[15] Goldman[16] considered stimulation or inhibition within ±10 percent of the controls to be insignificant. The following criteria were employed for the interpretation of the effect on algal response:

1. Significant stimulation—values ≤110 percent of the control.
2. No significant effect—values >90 percent <110 percent of the control.
3. Significant inhibition—values ≤90 percent of the control.

SUPPLEMENTAL PHOSPHORUS, NITROGEN, AND INORGANIC CARBON

In order to discount the possibility of stimulation of cultures due to supplemental phosphorus (P) or nitrogen (N) from the organic fractions, the effect of the supplemental P and N on algal cultures was investigated. The various fractions resulting from gel chromatography were analyzed for phosphorus and nitrogen. Table I shows the amount of each organic fraction (Lake George effluent) which was added to the 100 ml of NAAM medium and the concentration of supplemental P and

* Provisional Algal Assay Procedures, 1969 medium revised in 1970.

N thus added to the medium as a result of the addition of these organic fractions. Table II gives similar information about the Clifton Knolls effluent organic fractions. Thus, P as K_2HPO_4 and N as $NaNO_3$, equal in amount to that being supplemented through the addition of an organic fraction, were added to the controls (see Tables I and II). Table IV contains the values of maximum specific growth rates (μ_{max}) and maximum standing crops for the controls as well as for cultures with supplemental inorganic P and N added to them. The values of μ_{max} and maximum standing crop have been expressed as percent of the controls.

The data in this table indicate that the supplemental P and N do not contribute to the stimulation of *S. capricornutum* as the values varied from 88 to 107 percent for μ_{max} and from 93 to 106 percent for the maximum standing crop. The fraction G-15-I and G-15-II are components of the fraction G-10-I, and similarly the fraction G-25-I is a component of the fraction G15-I. Since this is so, the content of P and N in each of these three fractions G-15-I, G-15-II, and G-25-I, will be less than that in fraction G-10-I. It was therefore unnecessary to investigate the effect of the supplemental P and N in these three fractions from each effluent. Porcella *et al.*[17] working with *S. capricornutum*, pointed out that a 5 percent PAAP [18] medium (that is, 30 μg P/l, 700 μg N/l) was too rich in nutrients for μ_{max} to be significantly affected by nutrient concentration. For *S. capricornutum*, they obtained half saturation constants of 20 μg P/l and 500 μg N/l. In one of their experiments, Porcella *et al.*[17] varied the concentration of P by a factor of 15 (40 to 600 μg P/l) and no difference in μ_{max} was obtained. Middlebrooks *et al.*[18] noted that the test alga *S. gracile* grew at a rate independent of the P and N concentration above 50 μg P/l and

TABLE III. New algal assay medium (1970).*

Compound	Concentration (μg/l)	Element	Concentration (μg/l)
Macronutrients			
$NaNO_3$	25 500	N	4 200
K_2HOP_4	1 044	P	186
$MgCl_2$	5 700	Mg	2 904
$MgSO_4\ 7H_2O$	14 700	S	1 911
$CaCl_2\ 2H_2O$	4 410	Ca	1 203
$NaHCO_3$	15 000	K	468
		Na	11 004
		C	2 143
Micronutrients			
H_3BO_3	185.640	B	33.000
$MnCl_2$	264.270	Mn	114.000
$Zn\ Cl_2$	32.700	Zn	15.000
$CO\ Cl_2$	0.780	Co	0.350
$Cu\ Cl_2$	0.009	Cu	0.003
$Na_2Mo\ O_4\ 2H_2O$	7.260	Mo	2.880
$FeCl_3$	96.000	—	—
$Na_2EDTA\ 2H_2O$	300.000	Fe	33.000

Notes: 1. $K_2H\ PO_4$ should be added last to avoid iron precipitation. 2. Resulting pH of medium equals 7.43.

* "Provisional Algal Assay Procedures, 1969 (the medium revised in 1970)" Joint Industry Government Task Force on Eutrophication, New York.

TABLE IV. Effect of supplemental phosphorus and nitrogen on *Selenastrum capricornutum*.

Supplemental P and N Added (same as in Organic Fraction)	Maximum Specific Growth Rate Per Day‡ μ_{max} (%)	Maximum Standing Crop‡,§ (%)
Control	100	100
P and N added (Same as in LG Concentrate)	88	93
P added (Same as in G-10-LG-II)*	98	106
N added (Same as in G-10-LG-IV)	107	107
N added (Same as in G-10-LG-V)	106	106
P added (Same as in CK Concentrate)	96	96
P added (Same as in G-10-CK-I)†	91	101
P added (Same as in G-10-CK-II)	90	101

* G-10-LG-II—Fraction No. II of the Lake George effluent from Sephadex G-10 column.

† G-10-CK-I—Fraction No. I of the Clifton Knolls effluent from Sephadex G-10 column.

‡ The values of μ_{max} and maximum standing crop have been expressed as percent of the controls.

§ Maximum standing crop estimated by absorbance values at 600 nm for 5-cm light path.

TABLE V. Effect of organic fractions of Lake George effluent on the growth of *Selenastrum capricornutum* (absorbance measurements).

Organic Fractions Added	Maximum Specific Growth Rate/Day (EPA, 1971 Method)		Maximum Specific Growth Rate/Day (Regression Analysis)		
	$\mu_{max}day^{-1}$	Percentage of Control	$\mu_{max}day^{-1}$	Percentage of Control	Coefficient of Correlation
Control	1.079 ± 0.338*	100	0.749	100	0.943
Lake George Concentrate	1.346 ± 0.296	125	0.848	113	0.903
G-10-LG-I† (>700)	1.278 ± 0.206	118	0.869	116	0.936
G-10-LG-II (430)	1.381 ± 0.300	128	0.887	118	0.961
G-10-LG-III (117)	1.178 ± 0.258	109	0.815	109	0.975
G-10-LG-IV (Undefined)	1.139 ± 0.114	106	0.815	109	0.975
G-10-LG-V (Undefined)	1.122 ± 0.216	104	0.851	114	0.958
G-15-LG-I (>1 500)	1.246 ± 0.186	115	0.809	108	0.930
G-15-LG-II (1 200)	1.230 ± 0.100	114	0.965	129	0.981
G-25-LG-I (>5 000)	1.354 ± 0.096	125	0.876	117	0.934

* Values of μ_{max} ± 2 standard deviations.
† Values in the parentheses indicate the apparent molecular weight (AMW) of that fraction.

300 μg N/l. The results obtained in this current study are in conformity with these findings.

Any inorganic carbon that may have been incidentally added to the algal cultures in these organic fractions also did not play any part in the algal growth response. This is clear from the following observations:

1. The inorganic carbon in the NAAM medium was 2.143 mg carbon/l (Table III). The cultures were continuously shaken and efficient gas exchange was maintained through the foam plugs. The pH in the cultures was never more than 8.3. In the Algal Assay Procedure Bottle Test [18] it is mentioned ". . . test flasks are normally incubated to facilitate free gas exchange at the air/water interface. Therefore, since atmospheric carbon dioxide is available, the test outlined cannot be used to demonstrate algal growth limitation by carbon in water."

2. The organic fraction G-10-CK-III did not show any effect on the growth response of this alga even though 1.3 mg carbon/l of supplemental inorganic carbon was added to the culture.[14] Miller and Maloney [7] reported no effect on *S. capricornutum* when 20 mg carbon/l (as $NaHCO_3$) was added to the Shagawa Lake water whereas 10 percent secondary effluent significantly increased the growth.

Thus, the supplemental nitrogen, phosphorus or inorganic carbon resulting from the addition of the organic fractions did not contribute to the stimulation of this alga. It is therefore apparent that any significant stimulation or inhibition of algal cultures in this investigation was due to organic compounds present in these fractions.

ORGANIC COMPONENTS FROM EFFLUENT FRACTIONATION

The stimulation of the algal cultures by the additions of the organic fractions may be manifested as an increase in the maximum specific growth rate (μ_{max}) or the maximum standing crop.

Saunders [19] noted that dissolved organic matter could be effective as substrate, accessory growth factors, chelators, or toxins. Provasoli [20] reported that most of the algal species he studied (147 out of 204) required vitamins. Even though the vitamin requirement for *S. capricornutum* has not been fully established, it is possible that this test alga may be able to satisfy this requirement through the addition of organic wastewater fractions.

Pinter and Viney [21] demonstrated 3-Indole-acetic acid and vitamin B_{12} to be constituents of domestic wastewater. Whereas vitamin B_{12} may serve as an essential requirement for some algae, 3-Indole-acetic acid has been shown to act as a growth substance for algae.[22, 23] Growth substances affect the growth rate. Pipes [9] reported that some of the organic compounds in wastewater accelerated the growth rate of algae even when adequate CO_2 was present.

Rebhun and Manka [24] reported "humic" substances to be 40 to 50 percent of the soluble organics in an effluent from a trickling filter plant. The molecular weight of humic substances may vary from 600 to 300 000.[25] The AMW of various organic fractions used in this investigation varies from less than 100 to more than 5 000. The presence of humic substances, therefore, is not unreasonable in a number of organic fractions.

There is growing evidence that humic substances stimulate algal growth.[10, 11, 26] Whereas Bender *et al.*[10] and Lange [26] attributed stimulation of algae due to the chelation by humic substances, Prakash and Rashid [11] did not consider stimulation entirely due to chelation. They reported that the humic substances acted as specific sensitizing agents and enhanced the permeability of the plant cell wall thus resulting in an increased uptake of nutrients. They also concluded that the positive stimulating effect of the humic substances was independent of nutrient concentration. Organic fractions, responsible for algal growth stimulation, could have acted either of the following capacities: growth substances, that is, substances which promote growth but without which growth is possible, such as 3-Indole-acetic acid; or essential nutrients such as vitamins or trace metals.

Whereas maximum specific growth rate is related to the concentration of the rate-limiting nutrient, maximum standing crop is proportional to the initial amount of the limiting nutrient. Growth substances would affect the maximum specific growth rate while a supply of vitamins or trace elements will affect the maximum standing crop. The results can be interpreted as follows:

1. If there is an increase in μ_{max} but the maximum standing crop is not affected, the organic fraction can be assumed to have supplied growth substances.

2. If there is no change in μ_{max} but the maximum standing crop is increased, the or-

TABLE VI. Effect of organic fractions of Clifton Knolls effluent on the growth rate of *Selenastrum capricornutum* (absorbance measurements).

Organic Fractions Added	Maximum Specific Growth Rate/Day (EPA, 1971 Method)		Maximum Specific Growth Rate/Day (Regression Analysis)		
	$\mu_{max}day^{-1}$	Percentage of Control	$\mu_{max}day^{-1}$	Percentage of Control	Coefficient of Correlation
Control	1.079 ± 0.338*	100	0.749	100	0.943
Clifton Knolls Concentrate	1.443 ± 0.138	134	1.045	139	0.980
G-10-CK-I† (>700)	1.353 ± 0.318	125	1.033	138	0.986
G-10-CK-II (310)	0.986 ± 0.466	91	0.761	102	0.937
G-10-CK-III (100)	1.055 ± 0.156	98	0.726	97	0.955
G-10-CK-IV (Undefined)	1.360 ± 0.310	126	0.988	132	0.969
G-10-CK-V (Undefined)	1.202 ± 0.318	111	0.692	92	0.912
G-15-CK-I (>1 500)	1.246 ± 0.126	115	0.976	130	0.977
G-15-CK-II (750)	1.151 ± 0.028	107	0.870	116	0.992
G-25-CK-I (>5 000)	1.603 ± 0.010	149	1.006	134	0.960

* Values of μ_{max} ± 2 standard deviations.
† Values in the parentheses indicate the apparent molecular weight (AMW) of that fraction.

TABLE VII. Effect of organic fractions on the maximum standing crop of *Selenastrum capricornutum*.

Organic Fractions Added	Maximum Standing Crop (Absorbance)*	Day of Occurrence	Percentage of Control (%)
	Lake George Effluent Fractions		
Control	1.142 ± 0.170	9	100
Concentrate	1.173 ± 0.182	9	103
G-10-I	1.280 ± 0.112	9	112
G-10-II	1.261 ± 0.130	8	110
G-10-III	1.222 ± 0.070	9	107
G-10-IV	1.210 ± 0.290	9	106
G-10-V	1.081 ± 0.198	8	95
G-15-I	1.172 ± 0.268	8	103
G-15-II	1.366 ± 0.018	9	120
G-25-I	1.147 ± 0.460	8	100
	Clifton Knolls Effluent Fractions		
Control	1.142 ± 0.170	9	100
Concentrate	1.548 ± 0.033	9	135
G-10-I	1.311 ± 0.012	8	115
G-10-II	1.292 ± 0.124	11	113
G-10-III	1.142 ± 0.128	10	100
G-10-IV	1.208 ± 0.192	8	106
G-10-V	1.123 ± 0.228	10	98
G-15-I	1.267 ± 0.066	9	111
G-15-II	1.285 ± 0.024	10	112
G-25-I	1.240 ± 0.087	7	109

* Absorbance at 600 nm for 5-cm light path; absorbance values ± 2 standard deviations.

ganic fraction may supplement/supply essential nutrients/micronutrients.

3. If the μ_{max} as well as the maximum standing crop are increased, the organic fraction may supply growth substances as well as nutrients.

4. If there is inhibition, the organic fractions may act as a toxin or antibiotic.

GROWTH RESPONSE

The effect of organic fractions on three algal species, namely *S. capricornutum* (a green alga), *Anabaena flos-aquae* (a blue-green, nitrogen-fixing alga), and *Microcystis aeruginosa* (a blue-green, non-nitrogen-fixing alga) was investigated. The effect on *S. capricornutum* is discussed below.

Effect of organic fractions. Tables V–VII and summary Table VIII indicate that the Lake George concentrate, G-10-LG-I (AMW >700), G-10-LG-II (AMW 430), G-15-LG-I (AMW >1 500), G-15-LG-II (AMW 1 200), and G-25-LG-I (AMW >5 000) showed significant effect (that is, 110 percent or more) on the maximum specific growth rate (μ_{max}). Similarly, in the case of the Clifton Knolls effluent an increase in μ_{max} was noticed for the Clifton Knolls concentrate, G-10-CK-I (AMW >700), G-10-CK-IV (AMW not defined), G-15-CK-I (AMW >1 500), G-15-CK-II (AMW 750), and G-25-CK-I (AMW >5 000). McDonald [5] and McDonald and Clesceri [6] investigated the effect of various organic fractions of wastewater effluent on *S. capricornutum*. They concluded that the concentrated effluent, G-50-I (AMW >30 000) and G-25-II (AMW >1 000) significantly increased the growth rate of *S. capricornutum*. Middlebrooks *et al.*[18] obtained significant increase in μ_{max} of *S. gracile* when wastewater effluent was added to Lake Tahoe water. Pipes [9] fractionated organic compounds in wastewater by an extraction process. He obtained six fractions and determined their effect on *Chlorella pyrenoidosa*. A maximum increase in growth rate was observed for the water soluble fractions. Bender *et al.*[10] reported stimulation of the growth rate of natural algae from an organic fraction from secondary wastewater effluent (mol wt 500 to 1 000) and attributed this to chelation of trace metals by organic compounds in the fraction.

TABLE VIII. Effect of organic fractions on growth of *Selenastrum capricornutum* (percentage of control).*

Organic Fraction Added	Apparent Molecular Weight (AMW)	Summary of Results: μ_{max} day^{-1} (EPA, 1971) (%)	Summary of Results: μ_{max} day^{-1} (Regression Analysis) (%)	Summary of Results: Maximum Standing Crop (ABS/5 cm) (%)
Lake George Fractions				
Control		100	100	100
Lake George (LG) Concentrate		125	113	103
G-10-LG-I	>700	118	116	112
G-10-LG-II	430	128	118	110
G-10-LG-III	117	109	109	107
G-10-LG-IV	Undefined	106	109	106
G-10-LG-V	Undefined	104	114	95
G-15-LG-I	>1 500	115	108	103
G-15-LG-II	1 200	114	129	120
G-25-LG-I	>5 000	125	117	100
Clifton Knolls Fractions				
Control		100	100	100
Clifton Knolls (CK) Concentrate		134	139	135
G-10-CK-I	>700	125	138	115
G-10-CK-II	310	91	102	113
G-10-CK-III	100	98	97	100
G-10-CK-IV	Undefined	126	132	106
G-10-CK-V	Undefined	111	92	98
G-15-CK-I	>1 500	115	130	111
G-15-CK-II	750	107	116	112
G-25-CK-I	>5 000	149	134	109

* Based on absorbance measurements.

In the experimentation reported herein, the value of μ_{max} for the control varied from 0.749 to 1.079 per day, the lowest values being obtained from the regression calculations. The values obtained by other researchers are: McDonald,[6] 0.99 per day (Basic ASM medium); Porcella,[17] 1.2 per day (PAAP medium). These slight differences in μ_{max} values could be caused by the different media used by these investigators.

From Table VIII, it is evident that the Lake George concentrate and fractions G-15-LG-I and G-25-LG-I did not affect the maximum standing crop even though an increase in μ_{max} was noticed. On the other hand, fractions G-10-LG-I, G-10-LG-II, and G-15-LG-II increased the maximum standing crop as well as μ_{max}. A maximum value for standing crop (120 percent) was obtained from G-15-LG-II (AMW 1 200). Similarly, in the case of the Clifton Knolls effluent (Table VIII) the fractions G-10-CK-IV and G-25-CK-I increased μ_{max} but had no effect on the maximum standing crop. But the Clifton Knolls concentrate and fractions G-10-CK-I, G-15-CK-I, and G-15-CK-II, showed positive effects on maximum crop simultaneously with a stimulatory effect on μ_{max}. However, G-10-CK-II did not show any effect on μ_{max} but exerted a positive effect on maximum standing crop. In this case, the maximum effect on standing crop was noted for the Clifton Knolls concentrate (135 percent). It may be pointed out that neither the concentrated effluent nor any organic fraction exhibited any inhibitory effect on any of the two growth parameters (μ_{max} and maximum standing crop). None of the organic fractions (of wastewater effluent) has been reported by McDonald [5] and McDonald and Clesceri [6] to indicate an inhibitory effect on *S. capricornutum*. The organic fraction G-10-III (AMW not defined) was reported by them to have indicated the lowest growth rate which was 98 percent of the control.

In the natural environment organic compounds present in wastewater effluents may, as well, act as chelators but in this study presence of Na_2 ethylenediaminetetracetic acid (EDTA) in the growth medium would more than likely limit their role in this capacity.

From the results discussed above it is observed that fractions G-10-LG-II (AMW 430), G-10-CK-IV (AMW not defined) and all fractions with AMW >700 showed stimulatory effect on μ_{max}. It may be concluded that high molecular weight fractions (AMW >700) in both effluents exerted positive effect on μ_{max}. These results are in accord with those of McDonald [5] and McDonald and Clesceri [6] who reported stimulation in growth rate of *S. capricornutum* by fractions of AMW >700.

The following possible explanations for these effects on μ_{max} can be advanced:

1. The concentration of low molecular weight fractions (G-10-III, G-10-IV, G-10-V) is small and their effect may not be markedly evident.

2. The cultures are nonaxenic. The bacteria may oxidize high molecular weight organic compounds and the resultant CO_2 could supplement the inorganic carbon.

3. The organic compounds sensitize the cells making the cell membrane more permeable [11] so that organic compounds/nutrients in the medium are able to penetrate more readily, thus stimulating the growth response.

With regard to the first explanation, it may be pointed out that the concentration of each organic fraction after its addition to the culture medium was the same as in the original unconcentrated wastewater effluent. This corresponds to a situation of a domestic waste discharge stream without any dilution. It is not anticipated that the concentration of an organic fraction higher than that in the effluent will ever occur in domestic waste. If no stimulation was observed at this concentration, it is unlikely that this particular organic fraction will stimulate algal growth at any lower concentration. Thus the low molecular weight organic fractions existing in wastewater effluents as typified by the wastewaters herein studied do not play any role in stimulation of the test alga.

Secondly, the NAAM medium, as discussed before, is not CO_2 limiting for *S. capricornutum*. Since these high molecular weight organic fractions had been subjected to bacterial oxidation during waste treatment processes, further oxidation of these organic compounds in the culture flasks is not very likely. These organic compounds, if not non-biodegradable, can be assumed to be difficult to oxidize. But if it is assumed that these high molecular weight organic fractions are oxidized and broken down into low molecular weight compounds in the culture flasks, no stimulation should be expected since low molecular weight fractions did not show stimulation in concurrent experimentation. These high molecular weight organic fractions also showed stimulation with carbon-14 uptake experiments [14] in which the organic fractions and carbon-14 were added daily to the culture flasks before incubation.

Lastly, the third explanation that the cells are sensitized and membranes become more permeable for these organic compounds to be effective seems plausible. However, the role of the organic compounds as chelators in the natural environment cannot be ruled out.

Regarding the effect on maximum standing crop, in both cases the fraction G-15-II with AMW 1 200 (for the Lake George effluent) and 750 (for the Clifton Knolls effluent) was significant (120 percent and 112 percent). There is a possibility that these fractions contain vitamin B_{12} (mol wt ≈ 1 000) which may be responsible for this increase in standing crop values. Similar increase in values is indicated by G-10-I (AMW >700) in both cases, while G-25-I (AMW >5 000) and G-15-LG-I (AMW >1 500) do not show any effect. These results give a further indication of the possibility of the existence of vitamin B_{12} in G-15-II. Similarly, the fractions G-10-LG-II (AMW 430) and G-10-CK-II (AMW 310) resulted in an increase in the maximum standing crop (110 to 113 percent). Observations [11] reporting an increase in the yield of marine dinoflagellates due to low molecular weight humic substances lends support to these observations.

CONCLUSIONS

1. Both Lake George and Clifton Knolls concentrated effluents showed significant stimulation of μ_{max}, whereas only the Clifton Knolls concentrate had stimulatory effect on maximum standing crop of *S. capricornutum*.

2. Some of the organic fractions singly resulted in greater stimulation of *S. capricornutum* than did the concentrated effluent.

3. No inhibitory effect on *S. capricornutum* of concentrated effluents or their organic fractions was noticed.

4. In general, the organic fractions with AMW >700 indicated stimulation of both μ_{max} and maximum standing crop for *S. capricornutum*.

5. No stimulation in growth response could be attributed to the supplemental nitrogen, phosphorus or inorganic carbon added to the medium from the addition of the organic fractions.

6. Stimulation of *S. capricornutum* was caused by the organic compounds in fractions. These compounds probably supplied growth substances as well as nutrients. In this study their role as chelators is doubtful due to the presence of Na_2 EDTA, but in the natural environment they may act as chelators.

7. Removal of nitrogen and phosphorus only from secondary effluent may not solve the problem of algal growth stimulation in receiving waters. The removal of organics of the nature of those herein reported in some instances, should be seriously considered in approaches to water quality improvement.

8. Specifically, the removal of organic components of AMW >700 should substantially decrease algal growth.

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