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Predicting the Algal Stimulatory Properties of Wastewater Effluent

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ABSTRACT

The Algal Assay:Bottle Test (AA:BT) was used to assess the relative roles of dissolved organic and inorganic sewage nutrients, contained in several municipal wastewater samples, in stimulating algal growth. The growth response of the green alga, Selenastrum capricornutum, to varying additions of either sewage or reconstituted water containing equivalent levels of inorganic nitrogen and phosphorus, was compared to algal growth levels calculated from the inorganic nutrient levels of these additions using the algal yield factor of the test alga. The additions resulted in a linear increase in algal growth with percent of sewage or chemical equivalent solution addition which was in close agreement to the values predicted from the total soluble inorganic nitrogen concentration in the algal cultures alone. The observation that organic nitrogen sewage fractions did not contribute directly to the observed algal growth; was partially attributed to the insufficient time of the AA:BT to permit mineralization of organic nitrogen compounds to utilizable inorganic forms.

PREDICTING THE ALGAL STIMULATORY PROPERTIES OF WASTEWATER EFFLUENT

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Discharge of wastewater effluent into the aquatic environment is of environmental concern for many reasons. One is the possible eutrophication, or rapid aging, of receiving waters. Various methods for predicting and assessing this impact have been developed. One approach is to calculate a predicted level of phytoplanktonic growth induced by the input of a nutrient-rich wastewater by examining the N:P ratio [equal to $(NH_3\text{-}N + NO_2^-\text{-}N + NO_3^-\text{-}N)/(\text{Ortho-P})$] of the receiving water and the nitrogen and phosphorus added by the wastewater input. It has been shown that nitrogen and phosphorus are taken up by the alga <u>Selenastrum capricornutum</u> in a ratio of approximately 11.3:1[1]. Miller, et al.[1] therefore defined a water to be nitrogen limiting for algal growth at an N:P ratio of less than 10:1, and phosphorus limiting for algal growth at an N:P ratio of greater than 12:1. The predicted level of algal biomass arising from additional nutrient input can then be calculated using equation one or two[1]:

$$\begin{array}{l}\text{Predicted algal}\\ \text{biomass (mg/l.) for}\\ \text{a nitrogen limiting}\\ \text{receiving water}\end{array} = \left[\left(\begin{array}{l}\text{Total Soluble Inorganic}\\ \text{Nitrogen (mg/l)}\end{array}\right)\right]\left[\begin{array}{c}\text{Algal Yield}\\ \text{Factor}\end{array}\right] \pm 20\% \tag{1}$$

$$\begin{array}{l}\text{Predicted algal}\\ \text{biomass (mg/l) for}\\ \text{a phosphorus limiting}\\ \text{receiving water}\end{array} = \left[\begin{array}{c}\text{Orthophosphorus}\\ \text{(mg/l)}\end{array}\right]\left[\begin{array}{c}\text{Algal Yield}\\ \text{Factor}\end{array}\right] \pm 20\% \tag{2}$$

where the Total Soluble Inorganic Nitrogen level (TSIN) is equal to NH_3-N + NO_3^--N + NO_2^--N. Algal Yield Factors for the alga Selenastrum capricornutum of 430 and 38 have been reported for phosphorus and nitrogen limitation respectively.[1] Alternatively, the Algal Assay: Bottle Test (AA:BT) has been cited quite frequently in the literature as a direct method for assessing the impact of a wastewater loading on the aquatic environment.[2-8] An excellent review of this procedure can be found in Miller et al.[1]

Dissolved inorganic nitrogen and orthophosphorus are the chemical species usually thought to be responsible for algal growth resulting from the addition of biologically treated municipal sewage to receiving waters since dissolved organic nutrient constituents generally comprise a very small percentage of the total nitrogen and phosphorus content of such wastewater[9]. If, however, a municipal wastewater effluent contains a larger percentage of organic nitrogen and phosphorus, then algal utilization of these components could lead to a growth response greater than that predicted by equations 1 and 2. Several investigators have examined the algal stimulatory properties of dissolved organic constituents of sewage. Middlebrooks, et al.[10] studied the algal growth response of Selenastrum gracile to additions of inorganic nitrogen and phosphorus in amounts equivalent to that of corresponding sewage effluent using Lake Tahoe (California) dilution water. With only one exception, the specific growth rates of the algae, in cultures containing wastewater additions, were greater than the response to additions of equivalent levels of inorganic nitrogen and phosphorus. Growth rate, however, has been suggested to be a poor growth parameter in batch cultures because it is indirectly related to external nutrient concentrations.[1] Other studies, such as by those of Sachdev and Clesceri[11] and McDonald and Clescari[12] have demonstrated the ability of certain organic wastewater fractions to stimulate algal growth. Additional work by Francisco and Weiss[13], Shapiro and Ribeiro[14], and Ferris, et al.[15], has shown algal growth to increase in direct proportion to the percentage of added wastewater and to be dependent upon the nutrient content of the receiving water, which varied seasonably.

The major objective of this study was to assess the accuracy of equations 1 and 2 in predicting the algal stimulatory properties of a wastewater discharge. This was accomplished using the AA:BT as a means of quantifying algal growth response. The study additionally sought to determine the relative roles of dissolved organic and inorganic sewage nutrients contained in several municipal wastewater samples in stimulating algal growth. This was done by comparing the growth response of *Selenastrum capricornutum* to varying additions of either sewage or reconstituted water containing equivalent levels of inorganic nitrogen and phosphorus dissolved in a buffered solution containing essential trace metals.

Approach

The biostimulatory potential of several municipal wastewaters was assessed by determining the algal response of receiving water to varying additions of the sewage, and comparing the results to those predicted by the inorganic nutrient content according to equation 1 or 2. The AA:BT was used in conducting the algal assays with

Selenastrum capricornutum as the test alga. The reliability of the AA:BT has been demonstrated by previous investigators for its ability to accurately predict the effects of wastewater upon algal growth in natural waters and to determine the primary limiting nutrient of receiving waters[3-8]. Several municipal wastewater effluents were tested during this first phase of the study including biologically treated sewage from Spencer, MA, Pittsfield, MA, and Amherst, MA. Dilution water for the Spencer and Pittsfield wastewater assays was collected from the receiving waters at points located just upstream from the point of discharge. Dilution water for the Amherst wastewater experiment was collected from the Mill River, in Amherst, MA. The nutrient status of the dilution waters was first established by observing the algal growth response of S. capricornutum to additions of nitrogen, phosphorus, $Na_2EDTA \cdot 2H_2O$, and trace mteals singly or in combination. The response of the test alga to varying percent additions of the three municipal wastewaters, in appropriate dilution water, was then determined.

The second phase of the study involved a comparison between the algal growth response of S. capricornutum to the varying percent sewage additions described above, vs. varying percent additions of solutions containing inorganic nitrogen and phosphorus levels equivalent to the sewage. The algal growth resulting from additions of the phosphorus and nitrogen sewage equivalents could then be used to assess the degree to which organic nitrogen and phosphorus sewage components were contributing to the biostimulatory properties of the sewage effluent according to the responses shown in Table 1.

TABLE 1

Interpretation of Algal Growth Response of *S. capricornutum* to Additions of Municipal Sewage or Inorganic Nutrient Levels Equivalent to the Sewage

Algal Growth Response as Measured by Maximum Standing Crop (mg dry wt/l)	Interpretation
1. Sewage additions = Equivalent inorganic nutrient additions = Theoretical yield from equation 1 or 2	Inorganic nitrogen and phosphorus species are the principal biostimulatory sewage components
2. Sewage additions > Equivalent inorganic nutrient additions and Theoretical yield from equation 1 or 2	Organic nitrogen and phosphorus components are contributing to sewage biostimulatory properties
3. Sewage additions < Equivalent inorganic nutrient additions and Theoretical yield from equation 1 or 2	Micronutrient limitation or heavy metal toxicity

Experimental Methods

The nutrient status of the river water, used as diluent in the sewage studies, was determined in accordance with EPA methodology[1]. Grab samples were collected from mid-width, mid-depth locations, in acid-washed glass bottles, packed in ice, and transported immediately to the laboratory. Portions of samples were autoclaved for 30 minutes at 121°C, to solubilize the bioavailable particulate matter, cooled, and re-equilibrated to atmospheric CO_2 levels by purging with a 1:99 CO_2:air mixture. Autoclaved and nonautoclaved samples were filtered through glass fiber filters and then through 0.45μ membrane filters to remove indigenous algae and other particulates from the water. The pH of the samples was readjusted to the *in situ* pH values by titration with either 0.5 N HCl or 0.5 N NaOH. Fifty ml volume subsamples of the autoclaved filtered water were transferred to 125 ml acid-washed and autoclaved Erlenmeyer flasks. One ml portions of 2.55 mg P/l stock phosphorus solution, 51.00 mg N/l stock nitrate solution, 51.00 mg/l stock $NA_2EDTA\ 2H_2O$ solution, and stock micronutrient solution were added to the water samples singly and in combination to give final concentrations described by Miller *et al.*[1] to identify the growth limiting nutrient(s). Algal inoculants were made from five to nine day old cultures of *S. capricornutum* grown in the algal nutrient medium described by Miller, *et al.*[1] The cultures were diluted and resuspended in filtered distilled water, and then delivered, in 1 ml portions, to each flask to give an initial algal concentration of about 1000 cells/ml.

Twenty-four hour composite sewage samples were collected, filtered, and added in varying percentages to autoclaved, filtered diluent water. Sewage samples were either collected prior to chlorination or were dechlorinated with sodium thiosulfate upon return to the laboratory. One mg/l $Na_2EDTA \cdot 2H_2O$ was additionally added to replicate samples to evaluate the possible presence of heavy metal toxicants or micronutrient limitation. S. capricornutum cultures were then added in 1 ml portions to each flask. Varying percent additions of a solution containing inorganic nitrogen (NH_4Cl and $NaNO_3$), phosphorus (K_2HPO_4 and KH_2PO_4), alkalinity ($NaHCO_3$), hardness ($CaCl_2 \cdot 2H_2O$) and pH (phosphate buffer) constituents equivalent to those present in the sewage were also added to diluent water and subjected to the AA:BT. Micronutrient additions were added to the reconstituted water to provide the same concentration of these elements as in the algal growth medium described by Miller et al[2].

Flasks containing sewage additions, chemical additions, or river water with nitrogen, phosphorus, EDTA, or micronutrient additions, singly or in combination, were incubated at 24°C ± 2°C under continuous cool white fluorescent lighting (400 ft. candles illumination) and kept in suspension by shaking at 100 oscillations per minute. Three replicate flasks were used for each treatment. The flasks were incubated for 14-20 days until the Maximum Standing Crop (MSC), measured as mg dry weight of algae per liter, was attained. The MSC was considered to have been achieved when the change in algal dry weight was less than 5 percent per day. Cell biomass was determined by evaluating mean cell volume, and cell numbers, using a ZBI Coulter Counter (Coulter Electronics, Inc., Hialeah, Florida) as described by Miller, et al.[1]

Both sewage and river water samples were analyzed for NO_3^--N, NH_4^+-N, NO_2^--N, organic-N, ortho-P, total-P, and pH. River water samples were additionally analyzed for hardness, alkalinity and dissolved oxygen. Chemical analyses were determined by various standard wet-chemical techniques.

Results and Discussion

The nutrient status of the dilution water, which was used in the sewage addition experiments, was first determined. The maximum standing crops of *S. capricornutum* in Quabog Pond, Housatonic River (Holmes Road Station) and Mill River (sampled on July 16 and September 27, 1981) dilution waters, containing additions of nitrogen, phosphorus, EDTA, and micronutrient solution, singly and in combination are shown in Figure 1. Corresponding chemical data are shown in Tables 1 and 2. The N:P ratios for these waters based upon chemical data were 1.7:1, 11.8:1, 8.3 and 5.3:1. respectively, indicating nitrogen limitation for all site excluding the Housatonic River water which was nitrogen and phosphorus co-limiting. Observed algal growth agreed with values predicted from the chemical content of the water using equation 1 or 2 with the exception of the September 27, 1981 sampling of the Mill River which displayed phosphorus limitation rather than the nitrogen limitation predicted by the measured N:P ratio of 5.3:1. The observation of phosphorus limitation in waters displaying a N:P ratio less than the usually accepted 10:1 value, has been previously reported[16]. Observed algal growth values were generally in the range predicted by equation 1 or 2 for samples containing chemical additions corresponding to the limiting nutrient(s) of the water. Algal growth in the autoclaved water aliquots were somewhat greater than the unautoclaved samples reflecting the probable solubilization of bioavailable particulate nutrients in the water during this step[1]. None of the waters displayed any micronutrient limitation or presence of toxicants.

TABLE I

Chemical data for Quabog Pond, Housatonic River (Holmes Road Station), and Mill River, dilution water[1] and Spencer, MA, Pittsfield, MA, and Amherst, MA municipal secondary wastewater.

Parameter	Site							
	Quabog Pond Dilution Water	Spencer Sewage	Housatonic River Dilution Water	Pittsfield Sewage	Mill River Dilution Water		Amherst Sewage	
					7/16/81	9/27/81	7/16/81	9/27/81
pH	8.0	-	7.7	7.5	6.3	6.5	6.8	7.0
Alkalinity (mg/l as $CaCO_3$)	-	-	-	-	6.82	19.40	35.96	61.90
Hardness (mg/l as $CaCO_3$)	-	-	-	-	18.56	17.50	84.54	84.50
Ortho-P (mg/l)	0.045	3.018	0.037	3.317	0.012	0.003	3.04	4.07
Total-P (mg/l)	0.054	3.056	0.052	3.615	0.025	0.029	3.24	4.72
NO_3^--N (mg/l)	0.041	3.969	0.030	15.558	0.100	0.012	11.50	10.50
NO_2^--N (mg/l)	0.012	0.019	0.006	0.017	0.000	0.000	0.25	0.23
NH_3-N (mg/l)	0.022	1.827	0.124	0.002	0.000	0.004	1.16	4.40
Total soluble inorganic-N (mg/l)[2]	0.075	5.815	0.434	15.577	0.100	0.016	12.91	15.13
Total organic-N (mg/l)	0.539	1.323	0.549	0.642	0.000	0.139	0.57	0.81
Percent Organic-N	88	19	56	3.9	0	90	4.2	5.1
Percent Organic-P	16.7	1.2	28.8	8.2	52.0	89.7	6.2	13.8
N:P	1.7	1.9	11.8	4.7	8.3	5.33	4.25	3.72
Predicted nutrient limitation[3]	N	N	N+P	N	N	N	N	N

[1] pH, alkalinity, and hardness were determined before autoclaving. All other parameters were determined after autoclaving.

[2] Total soluble inorganic nitrogen = NH_3-N + NO_2^--N + NO_3^--N.

[3] Predicted limitation: N:P<10, nitrogen; N:P>12, phosphorus; N:P 10-12, co-limitation.

TABLE 2

Maximum Standing Crop (MSC) of S. capricornutum (mg dry weight algae/l) in cultures containing additions of nitrogen, phosphorus, EDTA, or trace metals, singly or in combination, and environmental dilution water.

Treatment[1]	Site[2]							
	Quabog Pond		Housatonic River		Mill River			
					7/16/81		9/27/81	
	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
Control, autoclaved	3.28	2.85	19.76	15.91	6.14	3.67	0.32	0.59
Control, unautoclaved	-	-	10.68	8.17	2.26	2.58	0.21	0.59
C+P	3.72	2.85	23.07	16.49	10.70	3.67	2.91	0.59
C+N	8.30	19.35	33.60	15.91	4.45	5.16	0.21	1.28
C+P+N	39.72	40.85	53.76	37.41	38.90	26.66	29.79	22.78
C+EDTA	3.59	2.85	22.20	15.91	5.50	3.67	0.20	0.59
C+P+EDTA	3.82	2.85	21.68	16.49	9.49	3.67	2.71	0.59
C+N+EDTA	9.36	19.35	37.10	15.91	9.49	5.16	0.22	1.28
C+N+P+EDTA	40.40	40.85	58.20	37.41	41.25	26.66	29.29	22.78
C+Micro-nutrients	-	-	20.01	15.91	8.65	3.67	0.27	0.59
Observed nutrient limitation[3]	N	N	N	N+P	N+P	N	P	N

[1] C = Control; +P = Plus phosphorus; +N = Plus nitrogen; +EDTA = Plus $Na_2EDTA \cdot 2H_2O$.

[2] Predicted value = value ±20% as calculated from equation 1 or 2, depending on nutrient limitation.

[3] *Observed nutrient limitation determined from the relative algal growth response in cultures receiving* nutrient additions, singly or in combination.

The chemical content of the sewage effluent is also presented in Table 1. All of the effluents were nitrogen limiting. With the exception of the Spencer wastewater, the effluent contained less than 5% organic nitrogen relative to the total nitrogen content. The percent organic phosphorus content was variable among the sewage effluents ranging between about 1 and 14% of the total phosphorus content. Values for the MSC of S. capricornutum, in mg dry wt of algae/l, for samples containing varying percent additions of secondary sewage collected from the Spencer, MA, Pittsfield, MA, and Amherst wastewater treatment plants and dilution water sampled from Quabog Pond, the Housatonic River (Holmes Road Station) and the Mill River, respectively, are presented in Table 3 and illustrated in Figures 2-5. Additions of varying percentages of the sewage effluents into appropriate dilution water resulted in increased algal yields for all dilutions within the range of values predicted by inorganic nitrogen according to equation 1. All solutions containing added sewage were nitrogen limiting since they displayed a N:P ratio of less than 10:1. EDTA additions did not result in increased MSC values indicating the absence of heavy metal toxicity. The data indicate a linear relationship between increased algal yield and percent sewage addition in all cases. Correspondence of the observed algal growth with that predicted by the inorganic nutrient content, as calculated by equation 1 or 2, supports the hypothesis that dissolved inorganic nitrogen and phosphorus constituents rather than dissolved organic nutrients, were the primarily contributing constituents to the algal growth stimulatory properties of these wastewaters. This is supported particularly by the observed and predicted algal growth data for the additions of Spencer wastewater to Quabog Pond dilution water. Organic nitrogen comprised close to

TABLE 3

Maximum Standing Crop (MSC) of *S. capricornutum* (mg dry wt algae/l) for additions of varying percentages of sewage effluent to environmental dilution water.

Percent Effluent	Site[1]							
	Spencer STP		Pittsfield STP		Amherst STP			
					7/16/81		9/27/81	
	Predicted Yield ± 20%	Observed Yield	Predicted Yield ± 20%	Observed Yield	Predicted Yield ±20%	Observed Yield	Predicted Yield ±20%	Observed Yield
0	2.85±.57	3.28	15.91±3.18	17.21	3.67±.73	6.14	0.59±.12	0.32
0+EDTA	2.85±.57	3.72	15.91±3.18	19.11	--	--	--	--
1%	5.02±1.00	6.05	21.03±4.21	27.98	--	--	--	--
1%+EDTA	5.02±1.00	6.88	21.03±4.21	26.79	--	--	--	--
5%	13.76±2.75	13.89	41.52±8.30	51.58	28.22±5.64	32.97	29.31±5.86	35.71
5%+EDTA	13.76±2.75	14.47	41.52±8.30	50.90	28.22±5.64	33.22	29.31±5.86	33.67
10%	24.66±4.93	22.42	67.12±13.42	83.11	52.65±10.53	58.42	58.04±11.61	57.66
10%+EDTA	24.66±4.93	25.40	67.12±13.42	85.37	52.65±10.53	54.45	58.04±11.61	62.41
15%	--	--	92.73±18.55	113.43	--	--	86.76±17.35	97.87
15%+EDTA	--	--	92.73±18.55	113.56	--	--	86.76±17.35	95.94
25%	--	--	143.94±28.79	166.25	--	--	144.21±28.84	139.20
25%+EDTA	--	--	143.94±28.79	165.27	--	--	144.21±28.84	142.40
30%	68.29±13.66	60.57	271.98±54.46	275.41	--	--	--	--
30%+EDTA	68.29±13.66	64.51	271.98±54.46	266.46	--	--	--	--
50%	111.91±22.38	103.44	--	--	248.13±49.63	205.70	287.84±57.57	246.53
50%+EDTA	111.91±22.38	102.85	--	--	248.13±49.63	227.78	287.84±57.57	248.67
70%	155.53±31.11	139.93	--	--	--	--	--	--
70%+EDTA	155.53±31.11	146.67	--	--	--	--	--	--
100%	220.97±44.19	188.72	--	--	--	--	--	--
100%+EDTA	220.9 ±44.19	204.17	--	--	--	--	--	--

[1] All treatments, with the exception of the 0 and 0+EDTA addition in the Pittsfield STP study, were nitrogen limited. Predicted values were calculated using equation 1, for nitrogen limitation, or equation 2 for phosphorus limitation.

20% of the total nitrogen content of this sewage. Algal utilization of this organic portion would have resulted in growth beyond that predicted by uptake of inorganic nitrogen concentration alone. Yet the observed algal growth response was well within the range of values predicted by the inorganic nitrogen content of the sewage.

In order to corroborate the hypothesis that inorganic nitrogen and phosphorus species represented the principal biostimulatory component of the secondary wastewaters tested here, two algal assays were conducted using solutions which contained inorganic nitrogen, phosphorus, alkalinity, hardness, and pH constituents equivalent to those present in the Amherst sewage, using Mill River dilution water. The materials and methods of this experiment have been previously described. Values of the maximum standing crop of S. capricoruntum, in mg dry wt of algae/l, in samples containing varying percent additions of the chemical equivalent solutions (CES) are presented in Table 4 and illustrated in Figures 4 and 5. The MSC values for actual sewage additions were presented previously in Table 3 and Figures 4 and 5.

The observed MSC values for the July 16, 1981 sampling, in all but the 21% addition of CES, were slightly higher than that predicted by equation 1. All solutions containing CES additions were nitrogen limiting. The regression line for this CES data displayed an r value of 0.9783. Statistical t tests, at the 99% confidence level, indicated that the EDTA additions had no significant effect on the MSC.

The observed MSC values for the September 27, 1981 CES additions were slightly greater than the values predicted by equation 1 at the 5% and 10% addition levels. MSC values at 15% and 25% additions were within the range of predicted values. Algal growth was nitrogen limited at all levels of addition. The regression line for this CES data displayed an

TABLE 4

Maximum Standing Crop (MSC) of S. capricornutum (mg dry wt algae/l) for additions of varying percentages of solution containing inorganic nitrogen, phosphorus, alkalinity, hardness, and pH constituents[1] equivalent to those present in Amherst wastewater using Mill River dilution water.

Percent chemical equivalent solution[5]	Solution equivalent to Amherst sewage sampled on July 16, 1981[2]		Solution equivalent to Amherst sewage sampled on September 27, 1981[3]	
	Predicted Yield[4] ±20%	Observed Yield	Predicted Yield ±20%	Observed Yield
0	3.67±.73	8.90	0.59±.12	0.11
2%	13.84±2.77	20.70	--	--
2%+EDTA	13.84±2.77	21.84	--	--
4%	23.89±4.78	33.29	--	--
4%+EDTA	23.89±4.78	38.87	--	--
5%	--	--	29.31±5.86	37.21
5%+EDTA	--	--	29.31±5.86	38.73
8%	44.02±8.80	58.04	--	--
8%+EDTA	44.02±8.80	67.27	--	--
10%	--	--	58.04±11.68	74.34
10%+EDTA	--	--	58.04±11.68	73.06
15%	--	--	86.76±17.35	103.51
15%+EDTA	--	--	86.76±17.35	113.42
21%	104.38±20.88	91.12	--	--
21%+EDTA	104.38±20.88	105.33	--	--
25%	--	--	144.21±28.84	142.80
25%+EDTA	--	--	144.21±28.84	163.84
50%	--	--	287.84±57.57	291.53
50%+EDTA	--	--	287.84±57.57	293.34

1-CES contained: $NaHCO_3$, $CaCl_2 \cdot 2H_2O$, NH_4Cl, K_2HPO_4, KH_2PO_4 in concentrations equivalent to the pH, hardness, alkalinity, and hardness levels of Amherst sewage, plus micronutrients ($CaCl_2 \cdot 2H_2O$, H_3BO_3, $MnCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, $NaMoO_4 \cdot 2H_2O$, $ZnCl_2$, $CuCl_2 \cdot 6H_2O$, $MgSO_4 \cdot 7H_2O$, $MgCl_2 \cdot 6H_2O$).

2-Mill River sampled 7/16/81 used as dilution water.

3-Mill River sampled 9/27/81 used as dilution water.

4-Predicted Yield = 38 x (TSIN) ± 20%.

5-Hardness and alkalinity components in the CES, for the 7/16/81 experiment were about one half the level of the Amherst sewage for the 7/16/81 sampling.

r value of 0.9971.

In both experiments, a linear relationship was observed between MSC and percent CES addition. Figures 4 and 5 illustrate the close correspondence between the observed algal growth response in solutions containing sewage additions with those containing CES additions. Further analysis of the September 27, 1981 data indicated that the algal growth responses to the CES or sewage additions were statistically different at the 99% confidence level, only at the 10% and 50% addition levels. In both of these cases higher algal yields were observed for CES additions than for sewage additions. These data further support the hypothesis that the algal response to the Amherst sewage additions was attributable to the dissolved inorganic nitrogen and phosphorus content of the wastewater rather than to dissolved organic constituents.

Results of both sewage and chemical equivalent solution additions to appropriate dilution water, as presented in Table 1, indicated that total soluble inorganic nitrogen and orthophosphorus were the principal biostimulatory components of the wastewater effluents tested. The growth response of S. capricornutum to additions of several municipal wastewaters was virtually equal to the algal growth in dilution water containing additions of solutions comprised of equivalent TSIN, ortho-P, alkalinity, and hardness content. Furthermore, the MSC's for both the sewage and CES additions were within the ranges of theoretical yields predicted from equation 1 or 2 for most dilutions. In the case of the nitrogen limited growth observed in samples containing Amherst sewage and Mill River dilution water, algal growth was completely accounted for by the TSIN content of the cultures. This was further corroborated by algal growth data from cultures containing equivalent inorganic nitrogen and phosphorus additions. It seems unlikely, therefore, that the Amherst wastewater contained other soluble algal stimulatory compounds at significant levels.

It should be noted that the AA:BT protocol may preclude portions of particulate algal stimulatory constituents in municipal sewage owing to the necessary filtration step required to remove indigenous algae and particulates that would interfere with the biomass determination using the Coulter Counter. An average particulate-P to total-P ratio, for biologically treated wastewaters (without chemical phosphorus removal) can be estimated by assuming a suspended solids effluent composition of about 30 mg/l having a C:N:P ratio of 1:.1:.03. The particulate phosphorus content would then be equal to about 0.9 mg/l. Particulate phosphorus, then, represents between 15 to 30% of the 3 to 6 mg/l Total-P usually contained in such biologically treated wastewater, with the larger portion being present in the soluble form. The dissolved algal stimulatory wastewaer components would therefore appear to be of greater significance. Young, et al.[17] demonstrated that virtually all of the soluble phosphorus and an average of 63% of the particulate phosphorus in four municipal wastewaters was ultimately available for algal uptake. The results further showed that the short-term (14 days) availability of soluble phosphorus was significantly greater than that of the particulate phosphorus. These data further support the hypothesis that dissolved inorganic nutrients were the principal biostimulatory components of the wastewaters tested.

The low percentage of organic nitrogen (equal to 4.2% and 5.1% of the Total-N for the July 16, 1981 and September 27, 1981 samplings, respectively) in the Amherst wastewater used in these algal assays should be considered in evaluating the significance of the data presented here. Actual dissolved total organic nitrogen levels in the sewage may have been slightly greater than the values determined by Kjeldahl-N analysis owing to the inability of this technique to determine organic nitrogen in azides, azines, hydrozones, oximes, semicarbazones, and nitrile, nitro, and nitroso forms[18]. Algal growth observed in solutions of Mill River water containing varying percent additions of biologically treated Amherst sewage was generally equal to or slightly less than that predicted by the inorganic nitrogen level of the sewage according to equation 1 (see Figures 4 and 5). Algal growth beyond that predicted by equation 1 or above that observed in the CES additions would have been expected if the organic-N sewage fraction was contributing to algal growth. Thus the organic-N fraction does not appear to have contributed, to any observable extent, to algal growth in these cultures. This was supported by the correspondence between observed algal growth to that predicted by the total soluble inorganic nitrogen levels alone in Quabog Pond water receiving varying additions of Spencer sewage effluent, despite the 20% total organic nitrogen content of this wastewater. These data indicate that TSIN appears to be an accurate method for predicting the algal stimulatory response of sewage additions to receiving waters under conditions of nitrogen limitation.

The finding that organic nitrogen did not contribute directly to the observed algal growth was further demonstrated by data from the additions of the chemical solution, containing inorganic nutrient levels equivalent to that of sewage samples, to algal cultures. In all cases, the algal growth response to the CES additions was equal to or slightly greater than the algal growth observed in the equivalent sewage addition. Some biological variability is expected and is considered in the 20% range of predicted growth levels presented in equations 1 and 2. Such a response would not have been expected if the organic-N fraction in the actual sewage had contributed to algal growth. Nor can the lower response of the sewage cultures be attributed to the presence of toxicants or micronutrient limitation since this possibility was precluded in the other algal assay studies. The finding that the organic nitrogen content of the sewage effluents tested did not contribute significantly to the observed algal growth may be partially explained as an artifact of the AA:BT itself. It appears that the test does not allow sufficient time for mineralization of the organic nitrogen to utilizable inorganic forms. The contribution of organic phosphorus to algal growth in these samples could not be assessed since dilution water containing sewage or CES additions were always nitrogen limiting.

While these studies indicate that the levels of organic nitrogen in the secondary treated municipal wastewaters studied here did not stimulate algal growth, additional studies using wastewater effluents containing larger organic nutrient levels are needed to further clarify the role of these compounds in stimulating algal growth.

Conclusions

The AA:BT is a useful research tool in evaluating the bioavailable nutrients in natural waters and wastewater effluents. The algal growth response in solutions containing varying additions of either biologically treated municipal sewage effluent or chemical equivalent solutions of the inorganic nutrient content of the sewage was in close agreement to levels predicted by the total soluble inorganic nitrogen concentration in the culture, calculated according to equation 1. These additions resulted in a linear increase in algal yield with percent of sewage or CES addition. The algal growth stimulatory property of the biologically treated municipal wastewaters tested here were predicted by the total soluble inorganic nitrogen levels alone after determining that the algal growth in solutions containing the sewage additions were all nitrogen limited. Further studies are needed, using wastewater of varying inorganic and organic nutrient content, in order to more completely identify wastewater components which contribute to eutrophication of receiving waters.

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Authors

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Figure Captions

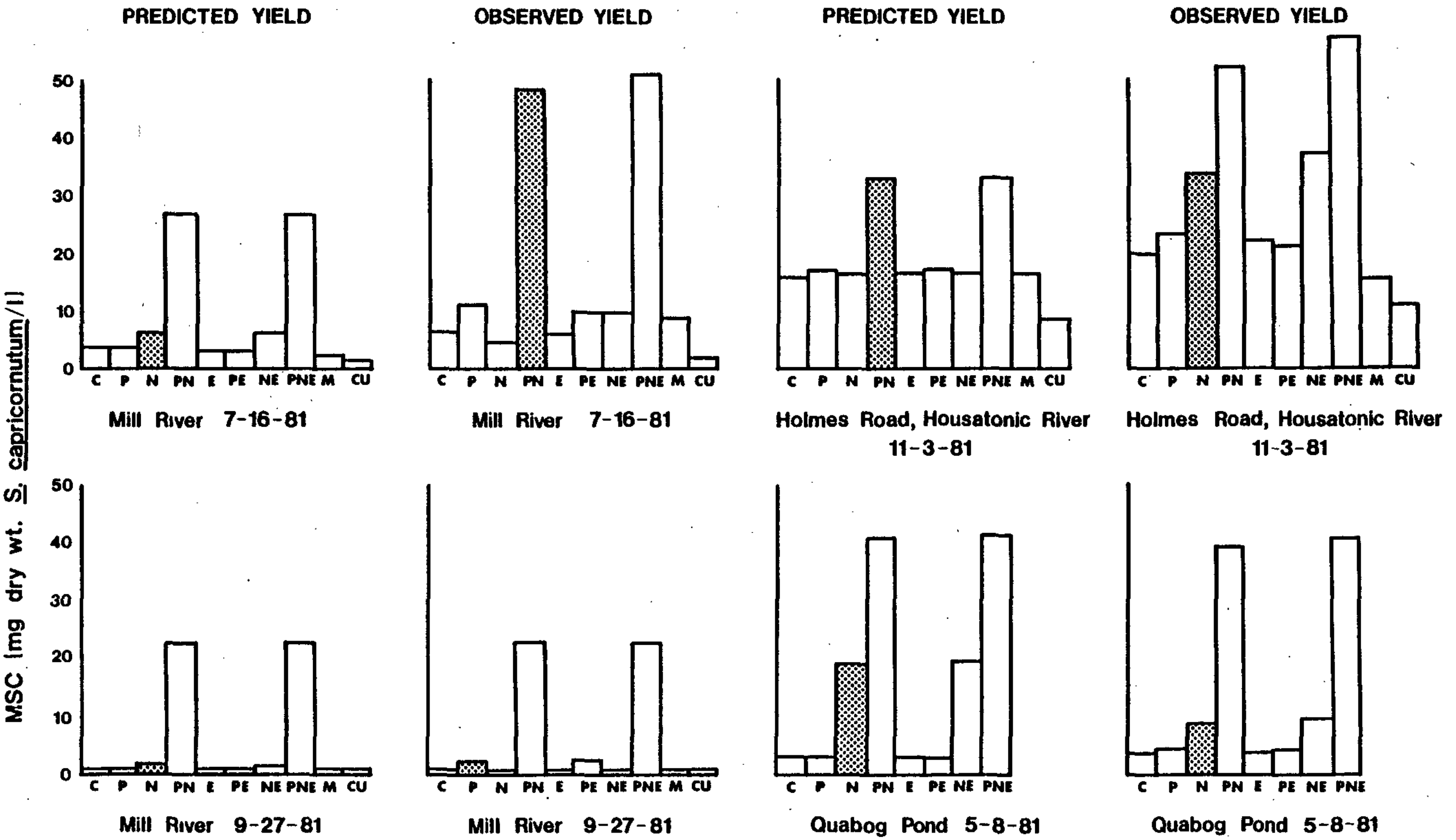
Figure 1: Predicted and observed yields (mg dry wt/l) of *Selenastrum capricornutum* grown in solutions containing additions of nitrogen (N), phosphorus (P), trace metals (M), and EDTA (E), singly or in combination. C = autoclaved contro, CU = unautoclaved control. Shaded histogram represents predicted or observed nutrient limitation.

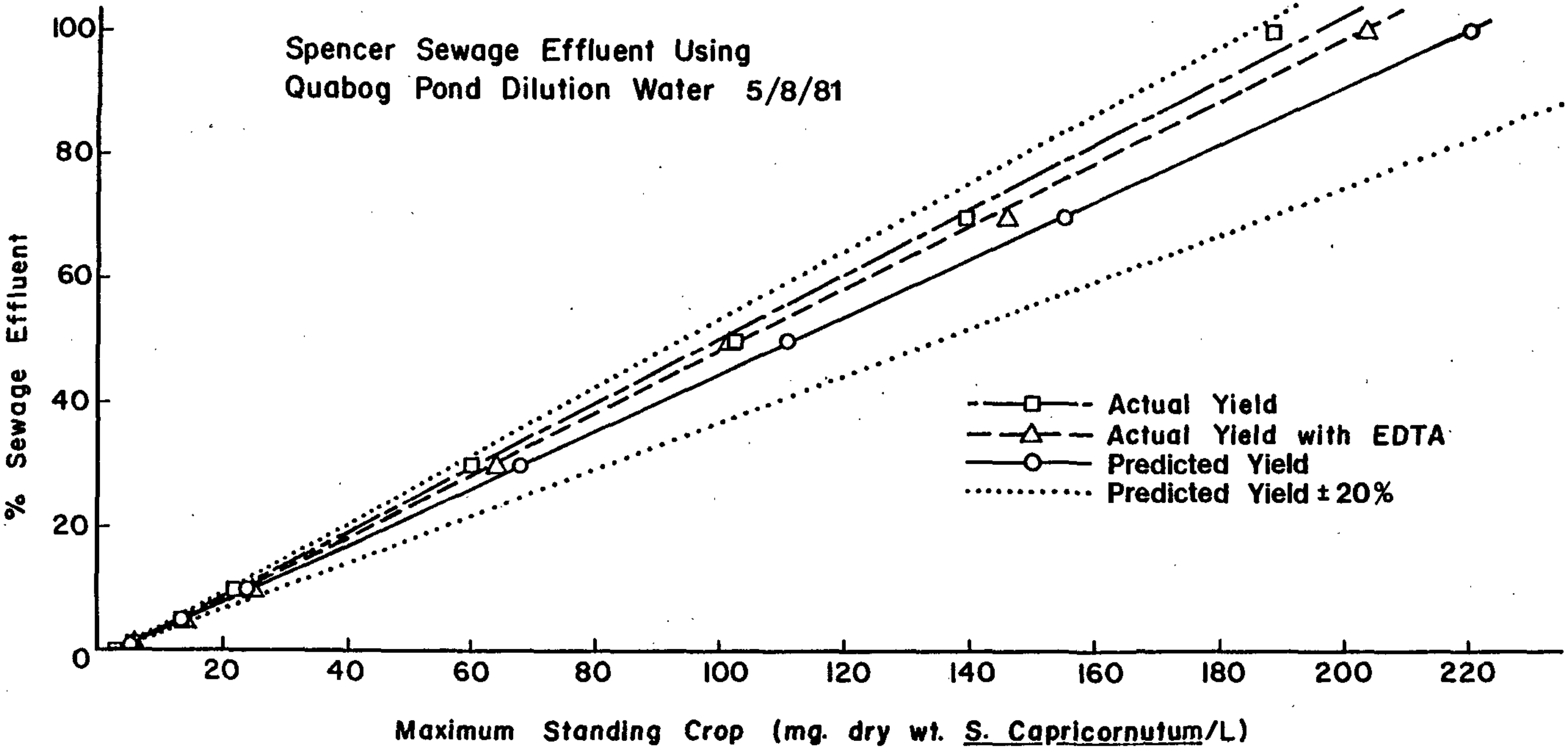
Figure 2: Predicted and actual (with and without EDTA) yields (mg dry wt/l) of *Selenastrum capricornutum* grown in Spencer secondary sewage effluent and Quabog Pond dilution water. Predicted Yield (mg dry wt/l) = 38 x TSIN (mg/l) ±20%.

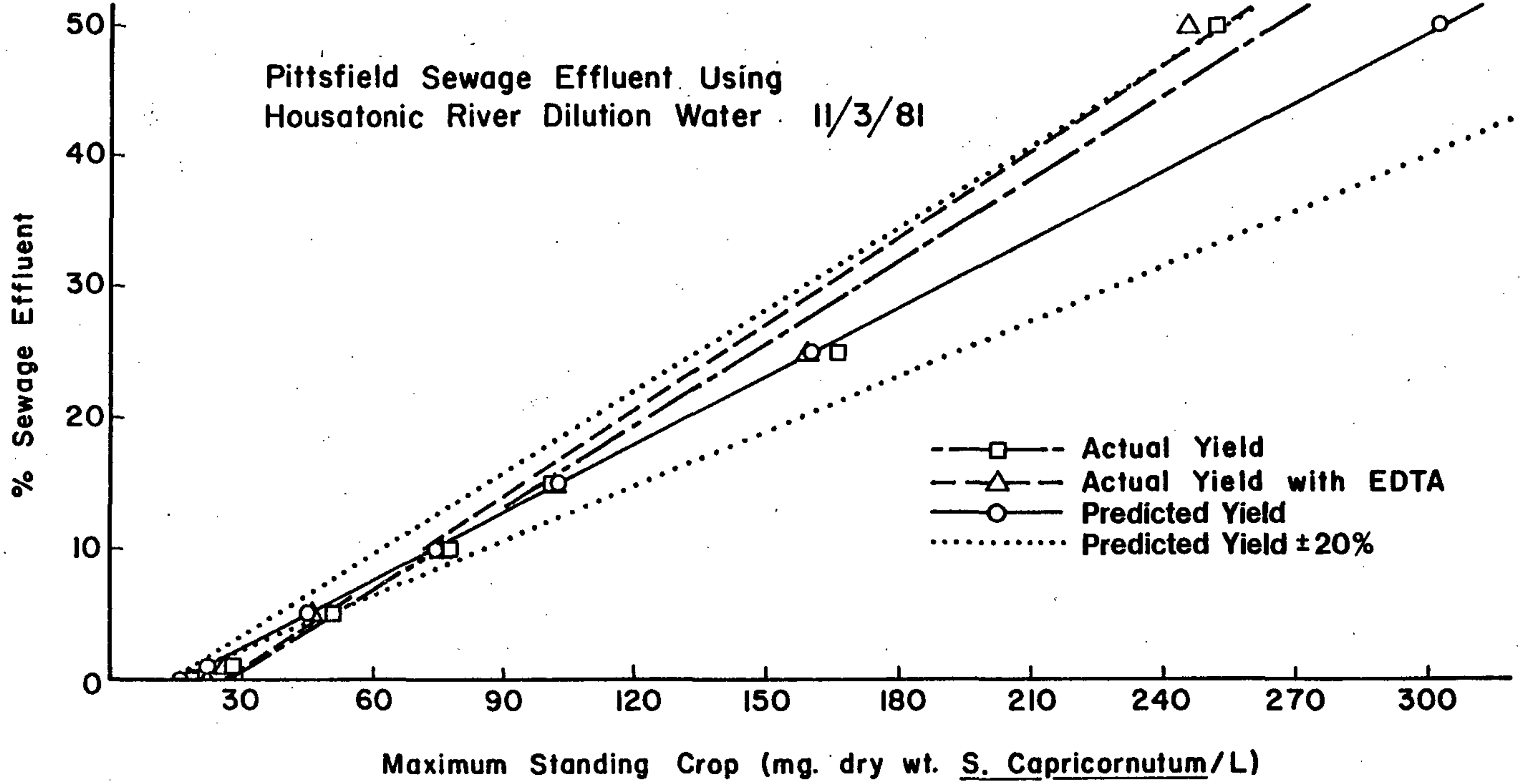
Figure 3: Predicted and actual (with and without EDTA) yields (mg dry wt/l) of *Selenastrum capricornutum* grown in Pittsfield secondary sewage effluent and Housatonic River dilution water (sampled at Holmes Road). Predicted yield (mg dry wt/l) = 38 x TSIN (mg/l) ±20% for all cultures containing sewage additions. Predicted yield (mg dry wt/l) for cultures containing dilution water alone = 430 x ortho-P (mg/l) ±20%.

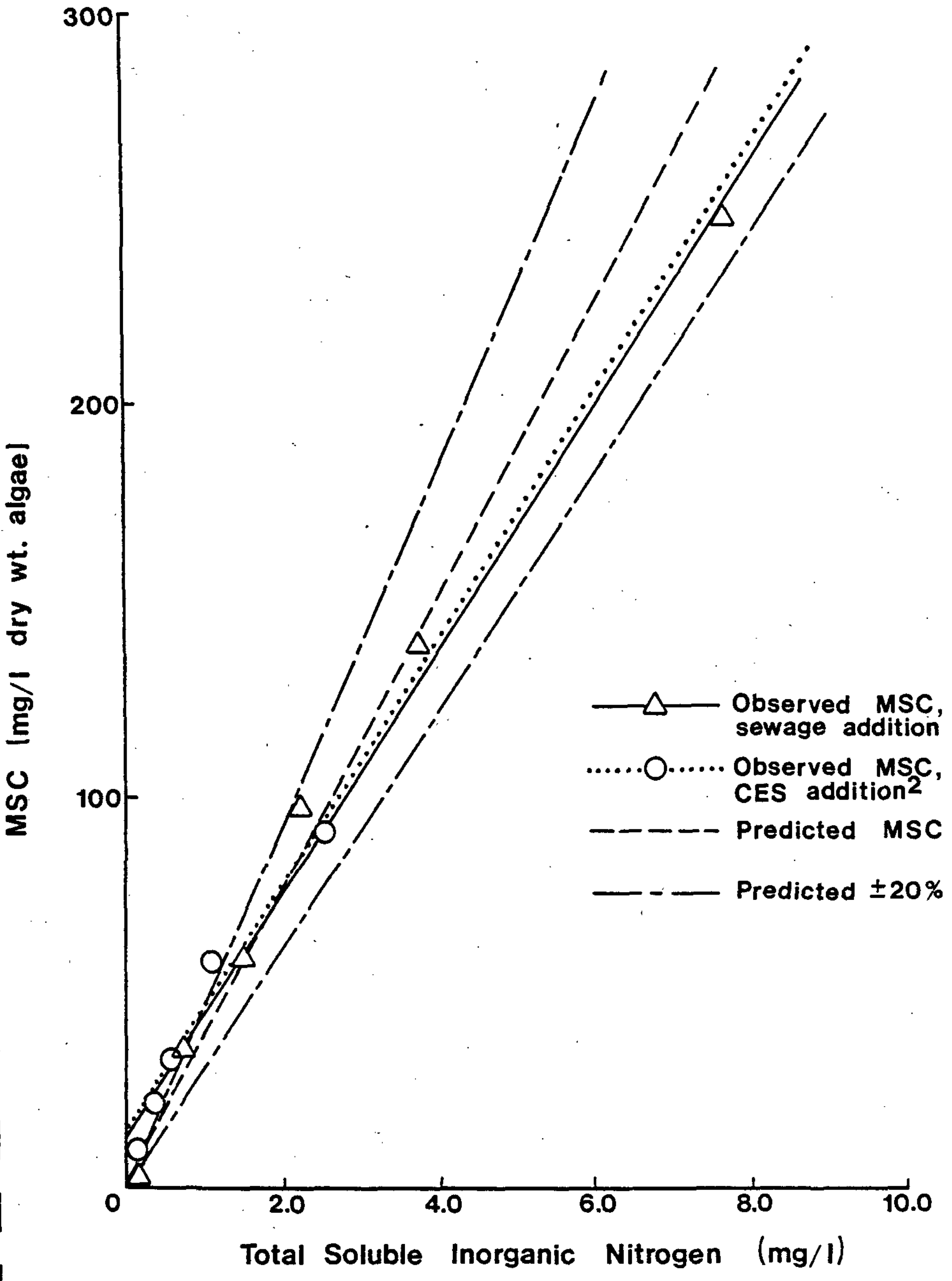
Figure 4: Observed and predicted yields (mg dry wt/l) of *Selenastrum capricornutum* grown in Mill River water, sampled July 16, 1981, plus Amherst secondary sewage or chemical equivalent solution (CES) additions. Hardness and alkalinity components in the CES were about one half the level of the Amherst sewage. Predicted Yield (mg dry wt/l) = 38 x TSIN (mg/l) ±20%.

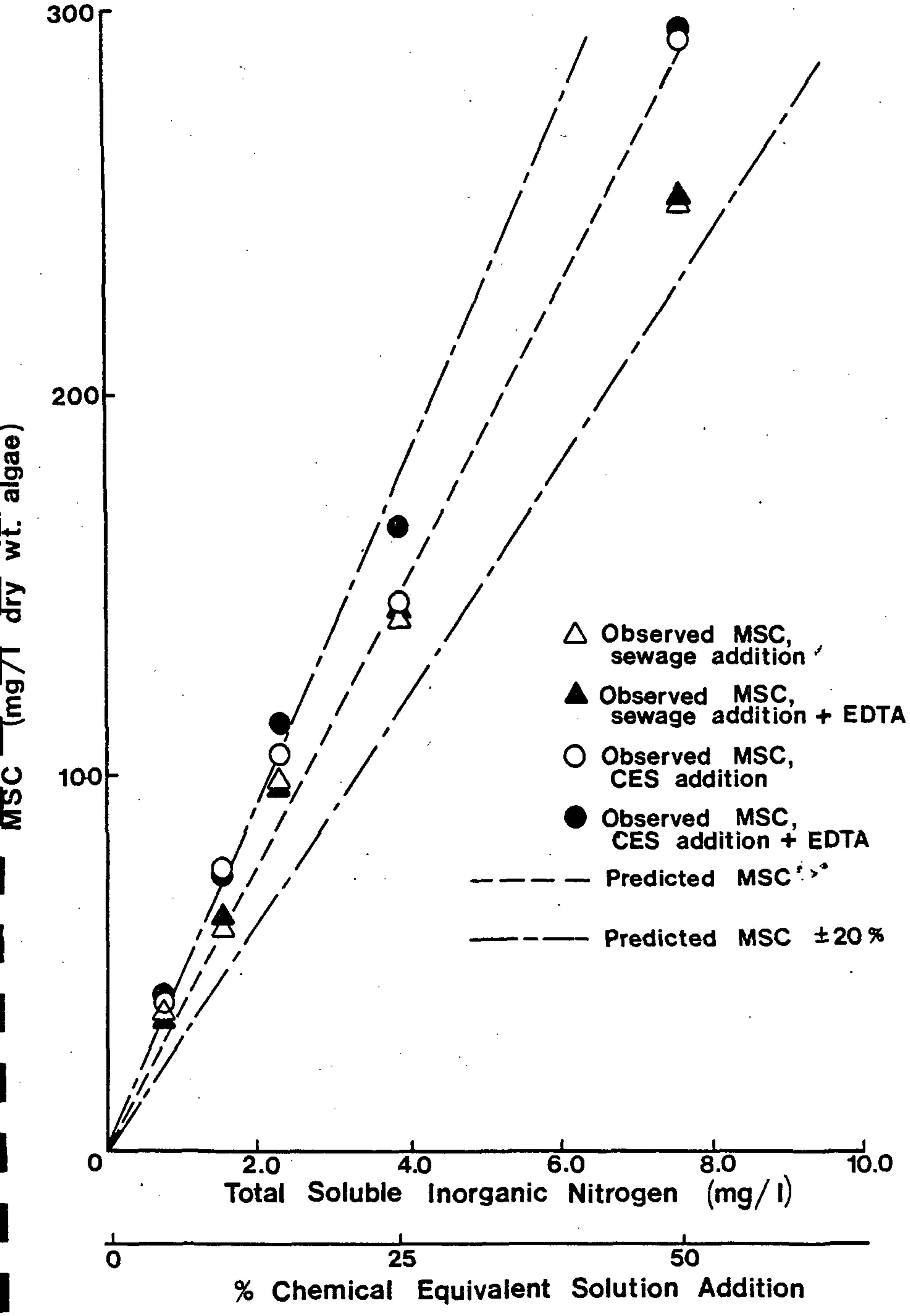
Figure 5: Observed and predicted yields (mg dry wt/l) with or without added EDTA, of *Selenastrum capricornutum* grown in Mill River water, sampled September 27, 1981, plus Amherst secondary sewage or chemical equilvalent solution (CES) additions. Predicted Yield (mg dry wt/l) = 38 x TSIN (mg/l) ±20%.


PREDICTED YIELD
OBSERVED YIELD
PREDICTED YIELD
OBSERVED YIELD
MSC (mg dry wt. S. capricornutum/l)
0
10
20
30
40
50
C
P
N
PN
E
PE
NE
PNE
M
CU
Mill River 7-16-81
Mill River 7-16-81
Holmes Road, Housatonic River 11-3-81
Holmes Road, Housatonic River 11-3-81
Mill River 9-27-81
Mill River 9-27-81
Quabog Pond 5-8-81
Quabog Pond 5-8-81


Spencer Sewage Effluent Using
Quabog Pond Dilution Water 5/8/81
% Sewage Effluent
0
20
40
60
80
100
20
40
60
80
100
120
140
160
180
200
220
Maximum Standing Crop (mg. dry wt. S. Capricornutum/L)
Actual Yield
Actual Yield with EDTA
Predicted Yield
Predicted Yield ± 20%


Pittsfield Sewage Effluent Using
Housatonic River Dilution Water 11/3/81


300
200
100
0
MSC (mg/l dry wt. algae)
2.0
4.0
6.0
8.0
10.0
Total Soluble Inorganic Nitrogen (mg/l)
Observed MSC, sewage addition
Observed MSC, CES addition^2
Predicted MSC
Predicted ±20%


300
200
100
0
MSC (mg/l dry wt. algae)
2.0
4.0
6.0
8.0
10.0
Total Soluble Inorganic Nitrogen (mg/l)
0
25
50
% Chemical Equivalent Solution Addition
△ Observed MSC, sewage addition
▲ Observed MSC, sewage addition + EDTA
○ Observed MSC, CES addition
● Observed MSC, CES addition + EDTA
Predicted MSC
Predicted MSC ±20%