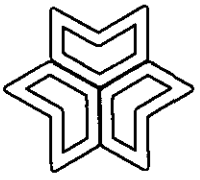

Environmental Engineering

Algal Growth Potential of
Secondary Treated Wastewater Effluent

by

Patricia E. Austin

January 1982

Department of Civil Engineering
University of Massachusetts at Amherst

Masters Project

Submitted in partial fulfillment of the requirements
for the Master of Science Degree in Environmental Engineering

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2/16/82

To

Lakes

Joe McGinn

Firmin

and return with
any comments to
Warren

- WJK comments
include a
pg with abbreviations

Table 11
(10) distinguish between
actual/predicted
yields

Qumsig
4/9

3/10

To Woo

Pg. 38 - Fig. 1 - Quabang Pond and Somerset WWTP not indicated on map.

Pg. 52 ¶ 1 line 2 - Samples were collected on 5/8 (see pg. 40) so how could they be analyzed on 5/7 ?

Introduction - ¶ 2 - hypolimnion

With my limited knowledge with algal assay, I found this report easy to comprehend and very informative. This could be very useful for Phos. justifications if someone is willing and able to do it. I would like a copy of this report for school (N.U.) project if I can get one

The Commonwealth of Massachusetts
University of Massachusetts
Amherst 01003

SCHOOL OF ENGINEERING
DEPARTMENT OF CIVIL ENGINEERING

July 6, 1982

Mr. Warren Kimball
MDWPC
P. O. Box 545
Westborough, Massachusetts 01581

Dear Warren:

Please contact me when you have time, concerning changes in the Algal Assay Report. Neil and I would like to finalize and print it as a technical report.

Thanks.

Sincerely yours,

Steve Plotkin

Steve Plotkin
Research Associate

SP:dbp

Algal Growth Potential

of

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Master's Project

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ABSTRACT

The biostimulatory properties of secondary treated wastewater effluent collected from the Amherst, Massachusetts wastewater treatment plant were evaluated using an algal assay technique. Algal assays indicated that the algal growth potential of Amherst wastewater could be determined from the inorganic nitrogen and orthophosphorus concentrations in the sewage. A chemical equivalent solution of the wastewater, containing equivalent orthophosphorus and inorganic nitrogen content, caused algal growth levels similar to those resulting from direct sewage additions. Phosphorus removal by alum or lime treatment significantly decreased the algal growth in additions of both sewage and chemical equivalent solution to Mill River water.

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CHAPTER 1

LITERATURE REVIEW

Introduction

Much of the literature dealing with the growth response of algae to wastewater discharges is lacking in specific information needed to properly evaluate the effect of such wastewater on receiving waters. The experimental method most often used for studies involves the exposure of algae to various concentrations of waste material. The resulting growth response is then used to assess the impact of the wastewater on the receiving stream. Often, however, little information is provided on the chemical composition of the wastewater. Rarely is there any discussion of possible synergism or antagonism between components of the waste material.

Algal assay has additionally been used to assess the nutrient status of a water by evaluating the algal response to single or multiple additions of the pure chemical compounds. These studies are also used to predict the effect of a discharge of known composition on a receiving water. For example, the effect of changes in a municipal wastewater loading on a receiving water might be predicted by first determining the nutrient status of the receiving water using algal assay technique. This involves the addition of phosphorous, nitrogen, and carbon, singly and in combination to determine the limiting nutrient of the water source. It appears that few studies have examined the varying algal response to sewage additions versus additions of equivalent levels

of inorganic nitrogen and phosphorus as that in the sewage. Such information is needed to evaluate the effectiveness of wastewater treatment practices aimed at removing inorganic nutrient constituents of wastewater.

Response to Nutrients

Miller and Maloney (1971) examined the effects of secondary and tertiary wastewater effluent on Selenastrum capricornutum. The results of their study are summarized in Table 1-1. Chemical analysis of the secondary and tertiary effluent was not reported.

Table 1-1. Effect of nutrient additions on growth of Selenastrum capricornutum.

Nutrient Addition	Burntside River Algal Biomass (mg/l dry wt.)	Shagawa Lake Algal Biomass (mg/l dry wt.)
Control	0.08	10.0
20 mg/l carbon	0.08	10.0
0.06 mg/l phosphorus	4.00	10.0
1.0 mg/l nitrogen	0.08	10.0
0.06 mg/l P + 1.0 mg/l N	20.00	30.0
10% tertiary effluent	0.08	11.0
10% secondary effluent	40.0	40.0

Algae grown in	cells/ml	mg/l dry wt.
tertiary effluent	5.0×10^3	0.08
tertiary effluent + 0.02 mg/l P	2.3×10^5	4.60
tertiary effluent + 0.06 mg/l P	6.0×10^5	12.00

It was concluded that the Burntside River was phosphorus deficient, depending upon the time of the year. Secondary treated wastewater stimulated algal growth to a much greater extent than did tertiary treated wastewater. Algae grown in tertiary effluent showed increased growth following phosphorus addition. It was therefore concluded that implementation of advanced wastewater treatment on the receiving water would retard eutrophication.

Gargas (1978) studied the effect of primary treated sewage, and biologically and chemically treated sewage on growth of Selenastrum capricornutum. He found that the potential production of the receiving water was increased with addition of sewage. Primary treated sewage had a greater stimulatory effect than biologically and chemically treated sewage. Table 1-2 presents response of algae to five sewage loadings. The algal growth parameter evaluated here is maximum specific growth rate u_m, defined as

$$u_m = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

where x_2 = biomass at the time t_2

x_1 = biomass at the time t_1.

Table 1-2. Chemical composition of primary treated and biologically and chemically treated sewage and its effect on growth of Selenastrum capricornutum.

Loading of sewage (%)	Total Nutrient Concentration				u_m	
	treated		Biologically, chemically treated		Primary treated	Bio. & chem. treated
	N(mg/l)	P(mg/l)	N(mg/l)	P(mg/l)		
0	0.730	0.005	0.730	0.005	0.22	0.22
1.5	0.898	0.041	0.815	0.009	0.24	0.27
3.0	1.065	0.077	0.900	0.017	0.28	0.30
6.0	1.400	0.149	1.076	0.034	0.72	0.49
9.0	1.735	0.221	1.262	0.051	1.04	0.72
12.0	2.070	0.292	1.410	0.062	0.88	0.61

Shapiro and Ribeiro (1965) performed a series of algal assay experiments to determine the effluent constituent which was stimulating algal growth. They developed two algal cultures for this study. One culture was made up predominately of blue-green algae, the second contained green algae. Varying amounts of effluent were added to algal cultures diluted with river water. The results of this algal assay are presented in Table 1-3.

Table 1-3. Growth of green and blue-green algae in river water with sewage additions.

% effluent in water	resultant PO_4-P conc. (mg/l)	green algae (mg/l dry wt.) 9 days	blue-green algae (mg/l dry wt.) 9 days
0	0.037	120	100
5	0.295	140	220
10	0.553	190	360
20	1.070	280	400
40	2.100	360	440

Addition of the wastewater stimulated growth of both the blue-green and the green algae. In order to determine whether the growth stimulating ability of the wastewater was solely attributable to phosphorus content, additional assays were performed using river water to which orthophosphate in amounts equivalent to that present in the wastewater were added in dilutions of 0, 5, 10, 20, and 40 percent. The results are presented in Table 1-4.

Table 1-4. Growth of green and blue-green algae in river water with additions of orthophosphate equivalent of sewage.

orthophosphate added in amt. equal to that if sample contained	green algae (mg/l dry wt.) 9 days	blue-green algae (mg/l dry wt.) 9 days
0% effluent	120	100
5% "	150	330
10% "	150	400
20% "	140	330
40% "	130	350

The experiment revealed the unsuprising result that growth by algal

species which cannot fix nitrogen becomes nitrogen limited in cultures containing increasing levels of added phosphorus. Alternatively, increasing phosphorus levels resulted in appreciable growth stimulation in blue-green algal species since they are capable of nitrogen fixation. This was corroborated by further studies, summarized in Table 1-5, which demonstrated that the factor necessary for green algal growth was not a trace element, but rather some macro nutrient.

Table 1-5. Growth of green and blue-green algae in river water with sewage additions, plus additional phosphorus spikes.

River water		green algae (mg/l dry wt.) 9 days	blue-green algae (mg/l dry wt.) 9 days
No addition		75	135
+ 5 % effluent		120	460
+ 5 % effluent + ortho-P to raise concentration to that of	10% ef.	140	530
	20% ef.	140	565
	40% ef.	140	540

To determine the major limiting nutrient to the green algae a fourth assay was run. Cultures were made up with river water and various combinations of $Ca(NO_3)_2$, $MgSO_4$, Na_2SiO_3, ferric citrate, and trace elements. The resulting chemical composition of the culture was not reported. Addition of these chemicals did not cause a significant increase in growth of green algae.

Another assay was performed on green algae using river water with added $Ca(NO_3)_2$. On the third day of the assay, phosphate was added. Table 1-6 summarizes the results of this assay.

Table 1-6. Growth of green algae in river water with $Ca(NO_3)$ and PO_4-P spikes.

River water plus	green algae (mg/l dry wt.)
no addition	70
$Ca(NO_3)_2$	80
$Ca(NO_3)_2$ plus PO_4-P added on Day 3	130

Shapiro and Ribeiro concluded that both groups of algae were stimulated by wastewater addition. Blue-green algal growth was stimulated by phorphorus while green algae required both phosphorus and nitrogen for growth stimulation.

This study additionally examined the effect of phosphorus and NH_4^+-N removal on algal growth. Phosphorus and nitrogen were removed from wastewater effluent by chemical precitation and NH_4^+-N stripping, respectively. Phosphorus and nitrogen concentrations before and after treatment and algal assay results of treated effluent dilutions are shown in Table 1-7 and Table 1-8, respectively.

Table 1-7. Phosphorus and nitrogen concentrations in treated and untreated sewage.

Effluent	PO_4-P mg/l	NH_3-N mg/l
Untreated	5.200	38.4
Phosphorous removed	0.071	38.4
Ammonia removed, phosphorous restored.	4.800	14.8

Table 1-8. Growth of green and blue-green algae in river water with treated wastewater additions.

River water plus	green algae (mg/l dry wt.) phosphate removed	green algae (mg/l dry wt.) ammonia removed	blue-green-algae (mg/l dry wt.) phosphate removed	blue-green-algae (mg/l dry wt.) ammonia removed
0 % effluent	60	70	60	70
5 % effluent	no value	130	80	590
10 % effluent	70	120	80	800
20 % effluent	40	90	80	730
40 % effluent	80	200	80	550

The authors concluded that PO_4-P removal controls growth of both algal groups while removal of NH_4^+-H controls green algal growth alone. Since environmental waters usually contain both of these algal groups, advanced wastewater treatment to remove phosphorus appears to be a more beneficial approach.

Middlebrooks et al. (1971) investigated the nutrient status of Lake Tahoe, California, and the effects of wastewater discharges on this receiving water using algal assay techniques. Selenastrum gracile was used in both batch and continuous flow culture studies. Specific or maximum cell growth rate was used to evaluate algal response. Specific growth rate is defined as the number of cells produced per day per total number cells. The investigators determined the growth rates using cell number data alone since no significant variation of cell size was observed among cultures.

Lake Tahoe water was found to have a significant variation in biostimulant properties throughout the year. The researchers thought

that this variation was attributable to variable climatic conditions in the drainage basin and also to mixing of the Lake Tahoe water. Variation in the diluent water was considered in subsequent studies.

Additions of effluents from five different types of waste treatment systems were made to the lake water. With only one exception, the growth response of the algae to wastewater was greater than the response to additions of inorganic nitrogen and phosphorus in amounts equivalent to that in the effluent. The algae also exhibited a greater growth response to primary or secondary treated effluents than to untreated wastes. Wastes which received biological treatment caused a greater growth response than mechanically treated wastes.

Middlebrooks et al. (1971) performed several experiments using chemostat algal cultures. The levels of inorganic nitrogen that limited growth were found to be very low. It was suggested that nitrate-nitrogen and nitrite-nitrogen are only an index of the substances which actually limit algal growth, and that when inorganic nitrogen is present in concentrations greater than 15 μg/l some other factor controls algal growth. Middlebrooks et al. (1971) additionally observed that neither the maximum growth rate attained in batch studies nor the initial rate in chemostat cultures was representative of rates in steady-state systems. It should be noted that the recommended parameter for evaluation of algal growth response in batch cultures is the maximum standing crop expressed as dry weight. Growth rate is indirectly related to external nutrient concentrations. Thus, phytoplankton exposed to equivalent nutrients may grow at

different rates (Miller, et al., 1978).

A number of studies have examined the role of phosphate-containing detergents in the eutrophication potential of wastewaters. Ferris, et al. (1974) studied the stimulatory effects of wastewater containing phosphate detergents versus wastewater containing only non-phosphate detergents. The test algae, Selenastrum capricornutum-Printz, was grown in lake water containing phosphorus in the range of 0.01 to 0.04 mg/l phosphorus. Various additions were made to the algal cultures. The additions were: 1) sewage effluent containing no detergents (total soluble phosphorus = 4.9 mg/l P), 2) sewage effluent containing non-phosphate detergent (5.0 mg/l P), 3) sewage effluent containing detergent phosphate (7.8 mg/l P), 4) sewage effluent in which nutrients were removed (0.4 mg/l P), 5) inorganic phosphorus additions equivalent to the phosphorus in 1, 2, and 3. A domestic sewage known to be free of detergent was used for the first treatment. A non-phosphorus detergent was added to this sewage to simulate an effluent produced by households using non-phosphate detergents for the second assay solution. Detergent containing phosphate was added to the domestic sewage for the third test solution.

All of the waste additions, with the exception of the treated sewage, caused an increase in the growth of Selenastrum capricornutum. The increased growth response seemed to be dependent upon the nutrient status of the receiving water. Thus, a greater increase in algal growth was observed when sewage was added to a lake having a

low nutrient level than when sewage was added to a relatively fertile lake water. The authors could not find a significant difference between the effect of sewage, containing phosphate detergents, as compared to the other untreated sewage additions. The growth of cultures which received phosphorus additions in amounts equal to the corresponding wastewaters did not stimulate as much growth as the wastewater additions. Thus, other nutrients must have been involved in the growth response. Predictions of algal response could therefore not be based on phosphorus content alone.

Francisco and Weiss (1973) also examined the stimulatory effects of wastewater effluent containing detergent phosphates. They found that algal growth increased in direct proportion to the percentage of untreated wastewater added to algal cultures. No difference in the algal growth response was observed, however, for wastewater additions with or without phosphate detergents at each level of treatment. Porcella et al. (1973) also examined the algal growth response to wastewater containing non-phosphate detergents. It was found that some factor other than nitrogen and phosphorus was limiting for the receiving water studied. No difference in the biostimulation of sewage containing phosphate detergents and sewage containing non-phosphate detergent was observed.

Sachdev and Clesceri (1978) investigated the ability of different molecular weight dissolved organic fractions contained in wastewater to stimulate the growth of Selenastrum capricornutum (Kutz). Secondary treated wastewater effluent from two treatment

plants was analyzed. The effluents were first membrane filtered and then concentrated by freeze-drying technique. The concentrated effluents were then separated into different apparent molecular weight (AMW) fractions on Sephadex gels. The organic carbon content of the wastewater effluent, concentrated effluent, and chromatographed fractions were analyzed to determine the percent carbon concentration for each effluent fraction. The effect of additions of varying organic fractions on algal growth was determined by algal assay technique.

The study concluded the following:

1. Concentrated effluent additions from both wastewater treatment facilities significantly stimulated the growth rate of the algae, but only one had significant effect on the maximum standing crop.
2. Additions of some organic fractions alone caused greater algal stimulation than whole concentrated effluent additions.
3. No inhibitory effects of the concentrated effluent or their organic fractions on algal growth were observed.
4. In general, the organic fractions with AMW greater than 700 caused stimulation of both maximum growth rate and maximum standing crop.
5. Algal growth could not be attributed to additional nitrogen, phosphorus, or inorganic carbon contained in the organic fractions, and thus stimulation was caused by organic compounds contained in the fractions.

Based on these observations the authors further concluded that removal of nitrogen and phosphorus from wastewater may not solve algal growth problems. Removal of organics should be considered, specifically those components with AMW greater than 700.

A study by McDonald and Clesceri (1979) also examined the stimulatory effects of organic compounds contained in wastewaters. An experimental procedure similar to that of Sachdev and Clesceri (1978) was followed. The authors of this study also found that some of the organic fractions of the effluent caused a significant increase in growth of Selenastrum capricornutum and Anabaena flos-aquae.

Response to Nutrient Additions in the Presence of Toxicants

Much of the algal assay literature describes experimental procedures where single or multiple chemical additions are added to algal cultures. Shiroyama et al. (1973) and Greene et al. (1975) studied the effect of nitrogen and phosphorus on growth of Selenastrum capricornutum. They reported a linear relationship between algal mass and the nitrogen and phosphorus levels in the algal culture. Thus, in the absence of toxicants and when all other essential nutrients are present the growth response of Selenastrum capricornutum can be predicted based upon nitrogen and phosphorus content. Waters which contain at least 0.010 mg/l orthophosphorus will yield 0.43 mg dry weight of algae per 0.001 mg p/l. Similarly, each 0.001 mg/l total soluble inorganic nitrogen will yield 0.038 mg/l mg dry weight algae. These relationships can

be used to predict the response of receiving waters to varying nitrogen and phosphorus additions. The presence of toxicants may by indicated if expected growth levels are not achieved.

Greene et al. (1975) performed a series of algal assays to assess the effect of various waste additions to a river water and reported that there was generally no correlation between maximum yield of Selenastrum capricornutum and increased calcium, magnesium, carbon, or alkalinity levels.

Miller et al.(1973) performed an algal assay to evaluate the effects of waste discharge on the Spokane River. This study investigated the impact of proposed treatment facilities upon algal growth. The results of some of the algal assays are shown in Table 1-9.

At the first site algal growth was greatly stimulated by addition of nitrogen plus phosphorus. However, growth was only seventy percent of the value predicted from nutrient analysis of the water. Failure to meet the expected yield here points to lack of essential nutrients and/or presence of toxicants. At the second site, the high zinc content seemed to preclude any stimulatory effect from nutrient additions. Zinc inhibition was evidenced at the third site as well. When the zinc was removed algal growth dramatically increased at the third site. Re-addition of zinc after metal removal caused growth to be inhibited again.

At the fifth site chelation of metals by EDTA additions caused a dramatic increase in algal growth. The large algal growth

Table 1-9. Response of Selenastrum capricornutum grown in treated and untreated waters to chemical additions.

Sampling site	Zn ug/l	Maximum 14 Day Algal Yield (mg/l dry wt.)							
		Control	+1000 mg N/l	+20 mg P/l	+N +P	Metals Removed	Metals Removed +100 mg Zn/l	Metals Removed +40 mg Zn/l	+1.0 mg/l EDTA
1. South Fork Coeur D'Alene at Mullan	18	0.030	0.042	0.310	8.20				
2. Spokane River at Post Falls	170	0.068	0.028	0.040	0.045				
3.Spokane R. at Seven Mile Bridge	75	0.120	0.061	0.055	0.050	15.40	0.032	0.020	
4. Spokane R. at Long Lake Dam	18	14.9	9.50	9.00	8.00				
5. Spokane R. at Riverside State Park		0.120							21.7

response in the control at the fourth site was indicative of the highly productive properties at this site. The lowered growth response from nitrogen and phosphorus additions indicates lack of essential nutrients or some toxic effects in those cultures. The authors felt that algal assay was an effective tool in studying nutrient enrichment problems which are complicated by the presence of heary metals.

Miller et al. (1975) performed a similar study on several rivers in the Spokane River System. Table 1-10 summarizes the results of these experiments. Algal growth at the first, second, and fourth sites was limited by phosphorus and nitrogen while algal growth at the third site was growth limited by constituents other than nitrogen or phosphorus. EDTA was added at the fifth and sixth sites to increase trace metal availability. The EDTA additions resulted in an increase in algal growth. The authors did not consider that the increased growth response may have been attributable to chelation of toxicants rather than increased trace metal availability. Removal of metals at the seventh site resulted in some algal growth. Re-addition of zinc to these cultures caused growth inhibition again. At the eighth site addition of EDTA caused a large increase in algal growth, pointing to zinc toxicity or micronutrient limitation.

Table 1-10. Response of Selenastrum capricornutum grown in treated and untreated waters to chemical additions.

Location	Algal yield (mg/l dry wt.)								
	Control	+1.0 mg/l N	+0.01 mg/l P	+0.05 mg/l P	+ 1.0 mg/l N + 0.05 mg/l P	+ 1.0 mg/l EDTA	Metals Removed	Metals Removed + 40 mg/l Zn	Metals Removed + 100 mg/l Zn
1. Snake River at Tildon Bridge	0.092	0.056	3.5	6.3	26.6				
2. Palouse R.	0.084	13.98	0.088	0.35	36.6				
3. Waldo Lake	0.043	0.043		0.056	0.045				
4. Snake River, Swan Valley	0.10	0.04	3.0	3.0	20.0				
5. Columbia R., Rock Island	0.30					5.4			
6. Columbia R., Bridgeport	0.10					4.5			
7. Seven Mile Road Bridge	0.11						14.0	0.02	0.032
8. Spokane R. Bowl and Pitcher State Park	0.12					21.7			

A discussion about the consequences of wastewater discharge on the algal growth in receiving waters would not be complete without some review of the potential inhibitory properties of chlorine on this phenomenon. Chlorine is commonly released to receiving waters as part of treated wastewater discharges. Chlorine is also released in cooling water discharges from power plants. Brooks and Liptak (1979) found that phytoplankton from Lake Michigan showed a significant chlorophyll loss and permanent decrease of carbon uptake rate after thirty minute exposure to total residual chlorine levels at or above 1.0 mg/l. Below this value only slight chlorophyll changes were evidenced and, following an initial decrease, carbon uptake rates exhibited nearly complete recovery.

Goldman and Quinby (1979) studied several marine algae for their ability to recover from chlorine stress. They found indigenous zooplankton and phytoplankton species capable of recovery after exposure to chlorine at contact times and contact levels typical of power plant discharges. These studies indicate that while there may be some temporary inhibition of algal growth response to chlorine inhibition, later decrease in chlorine concentration owing to the instability of this chemical species, may result in a subsequent algal growth response.

Summary

In summary, all the studies discussed in this review have involved the use of algal assay technique to study the response of algal species to single chemicals, chemical mixtures, or complex

waste materials. Algal growth has been shown to be stimulated or inhibited by addition of such compounds. A variety of parameters, such as inorganic nutrient content, presence of trace metals, and different organic fractions has been used as a first estimate in predicting the algal response to a wastewater input.

Little work has been directed towards comparing the algal growth response to an addition of one or more components of the wastewater. This is a complex problem because wastewater is comprised of many chemicals. There is a great potential for interactions between components in the wastewater as well as interactions arising from varying environmental and physical parameters, such as flow patterns and non-point source inputs. The problem is further complicated because the composition of wastewater is usually variable. The response of algae to stimulants or toxicants additionally is often species specific, and is also related to the chemical composition of the receiving water.

It is therefore very important to evaluate possible synergistic or antagonistic reactions between wastewater components and receiving water constituents which are encountered when the waste is discharged. Such information would provide a better evaluation of laboratory assay results with regard to field applications.

Only a limited number of studies, such as those by Middlebrooks et al. (1971), Ferris et al.(1974), Sachdev and Clesceri (1978), and McDonald and Clesceri (1979) have investigated specific components

of wastewater, such as inorganic nitrogen and phosphorus and organic molecular weight fractions, for their ability to stimulate algal growth. These studies all indicated that algal growth is greater than that observed for equivalent inorganic nutrient additions alone. Organic molecular weight fractions were additionally shown to stimulate algal growth. Such information is critical in designing advanced wastewater treatment methods to prevent such eutrophication of receiving waters. The study presented here further expands the information on this question by investigating the algal growth response to wastewater effluent and chemical additions equivalent to that present in wastewater, and comparing these results to growth values predicted by the chemical composition of these additions. Such information further identifies the growth stimulating components in wastewater effluent.

CHAPTER 2

MATERIALS AND METHODS

Introduction

The process of eutrophication of a water body depends upon a complex interaction of biological, chemical, and physical factors. It is therefore difficult to predict the effect of changes in nutrient loading on a receiving water from water quality analysis alone.

Algal assay has been developed as a tool to study the reactions within water bodies which stimulate planktonic growth. Algal assay, in conjunction with some other chemical analyses, may be used to predict changes which may occur in a receiving water attributable to an input of wastewater of variable nutrient composition. Algal assay may additionally be used to monitor the quality of lakes and streams.

Algal assay was used in this study to determine the algal growth components contained in wastewater. Inorganic nitrogen and orthophosphorus are the chemical species usually thought to be responsible for algal growth. Other chemicals contained in sewage effluent, however, may stimulate growth. Determination of the factors critical to algal growth is necessary for the proper design of wastewater treatment facilities.

A series of algal assay experiments were therefore performed to evaluate the response of river water to secondary treated wastewater effluent. The goal was to identify some wastewater components responsible for algal growth stimulation. The nutrient status of the

receiving water which was used as the diluent in these studies was first established. The river water was analyzed chemically and then subjected to algal nutrient limitation studies. The effect of sewage and other additions was then evaluated.

Secondary treated wastewater and inorganic nitrogen and phosphorus compounds in amounts equivalent to that in the sewage were added to river water to assess the factors causing algal growth. Observed algal yields were compared to theoretical yields. Determination of theoretical algal yield is based upon phosphorous and nitrogen content of the additions and the diluent. Algal growth resulting from sewage additions also was compared to growth resulting from additions of phosphorus and nitrogen equivalents of the sewage.

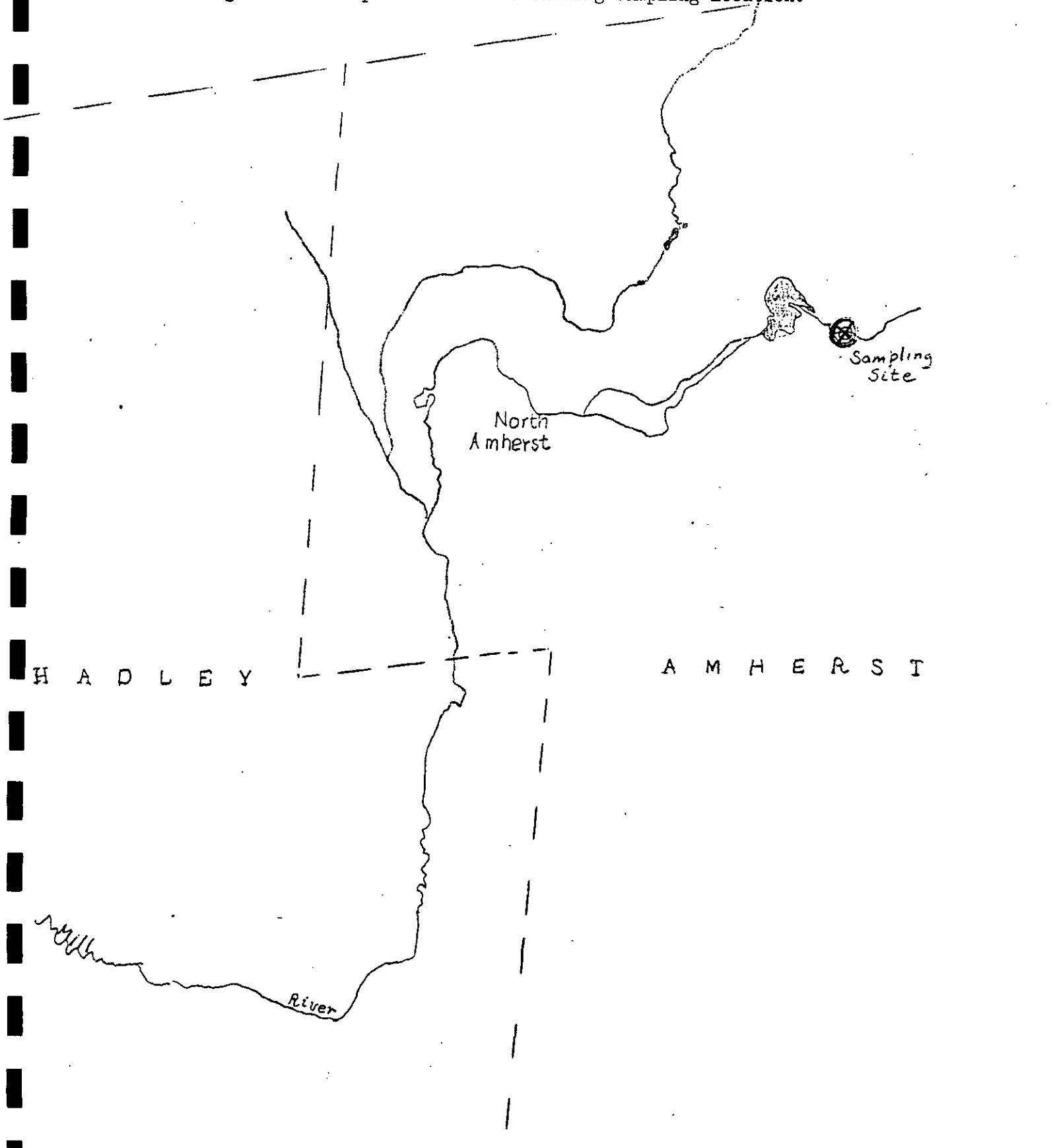
Secondary effluent was treated with either lime or alum to simulate tertiary treatment. The resulting algal growth was compared to theoretical growth values, based upon phosphorus reduction.

Sampling

The Mill River was sampled four different times during the study. Water was collected by grab sampling from a site directly upstream of the State Street bridge in North Amherst, as shown in Figure 2-1. The upstream site was chosen to minimize possible inputs arising from roadway runoff. The area of the Mill River sampled was known to be very clean and therefore provided good dilution water for the algal assay studies.

Water was sampled at mid-depth from an area in which the river was free flowing. The depth of the river was fairly shallow throughout

Figure 2-1. Map of Mill River showing sampling location.



the study, averaging approximately one foot in depth. The sample water was collected in pre-acid-washed, one gallon glass containers. Temperature and dissolved oxygen were recorded at the time of sampling. Since the sampling site was within a ten minute drive of the laboratory, it was not necessary to place the samples on ice.

Sample Processing

Upon return to the laboratory, pH, alkalinity, and hardness were immediately determined. The water was then autoclaved at 15 to 17 psi and 210° to 230° F. for a period of one hour. The period of autoclaving was longer than the recommended 30 minutes at 250° F. (Miller et al., 1978) since the operating range of the autoclave was less than the recommended temperature.

The samples were autoclaved to destroy all indigenous algal species. This is necessary to ensure the predominance of a single algal species during the algal assay. The autoclaving step additionally solubilizes nutrients present as particulates in the water. In theory, these nutrients would become available to algae in a natural environment over longer time periods. A non-autoclaved aliquot of sample was reserved and later chemically analyzed and subjected to algal assay to determine the effect of autoclaving on the dilution water.

After removal from the autoclave, the sample was allowed to cool to room temperature. It was then equilibrated with a 1% mixture of CO_2 and air to resaturate the water with CO_2. The pH was then adjusted to the original pH value obtained immediately after sampling. The sample was filtered through a 0.45 um membrane filter to remove

particulate matter and then stored in the dark at 4^{o} C. for later analysis.

A 24 hour composite sample was collected from the Amherst wastewater treatment plant. The plant is an activated sludge treatment facility which treats an average of 5 MGD. The sample was returned to the laboratory for pH, alkalinity, and hardness determinations. The effluent was then filtered through a 0.45 um membrane filter to remove indigenous algae and other unwanted biological contaminants (Miller, et al., 1978). It should be noted, however, that observed algal growth yields determined by algal assay may underestimate actual algal growth in environmental waters receiving wastewater effluent due to the sewage filtration. Young et al. (1980) have shown that particulate phosphorus does contribute to algal growth. Removal of this nutrient component prior to algal assay therefore results in lower observed yields. The percentage additional algal growth in actual environmental waters as compared to laboratory algal assay results would vary with the percentage of particulate phosphorus in the total phosphorus contained in the sewage.

Chemical Analysis

The following analyses were carried out on the sample water and the sewage effluent.

pH: pH was determined using a Fisher model 140 A Acumet pH meter.

Alkalinity: Total alkalinity was determined by titration of sample with 0.02 N HCl to pH 4.5 (U.S. EPA, 1979).

Hardness: Hardness was determined by titration of sample with 0.01 M Na_2EDTA using Eriochrome Black T as an indicator (U.S. EPA, 1979)

Nitrate, nitrite: Nitrate-nitrogen and nitrite-nitrogen were determined using the cadmium reduction method described by EPA (1979).

Total organic nitrogen: Total organic nitrogen was determined by converting organic nitrogen to ammonia using Kjeldahl digestion, followed by ammonia analysis (Strickland and Parsons, 1972).

Ammonia: Ammonia was determined using the scaled down Indophenol method described by Ram (1979), and based upon Strickland and Parsons (1972).

Total phosphate: Total phosphate was determined by boiling a sample aliquot with 11 N H_2SO_4 and 0.2 g $K_2S_2O_8$ to convert all the phosphorus to orthophosphate, followed by orthophosphate analysis. This method is based on phosphate analyses presented by EPA (1979) and Standard Methods (1980).

Orthophosphate: Orthophosphate was determined by the ascorbic acid method described by Strickland and Parsons (1972).

Algal Assay

The test alga used in this research was Selenastrum capricornutum-Printz, obtained from the EPA laboratories in Corvallis, Oregon. A 1.0 ml innoculum containing approximately 50,000 cells was pipetted into 125 ml erlenmeyer flasks, each containing 50 ml of test solution. The flasks were fitted with foam plugs to allow CO_2 and air exchange. The cultures were kept under 400 $\pm$ 10 % ft-c of fluorescent lights for 24 hours a day and continuously shaken at approximately 100 rpm. The laboratory set-up for the algal cultures is shown in Figure 2-2. Cells were grown until a maximum standing crop (MSC) was attained (14 to 21 days). MSC was considered to have been achieved when the change in algal dry weight determined on two consecutive days was less than 5 percent (Miller et al., 1978).

Cell number and mean cell volumes were determined using a model 5615_{ZB1} Coulter Counter with Model MHR MCV/Hct/RBC computer. The MSC was determined using the following formula:

$$\underset{(mg/l\ dry\ wt)}{MSC} = (Cell\ No.) \times \left(\frac{Mean\ Cell}{Volume}\right) \times \left(\frac{Specific\ Growth}{Coefficient}\right) \quad (1)$$

Specific growth coefficient was determined in previous laboratory work to be 3.6×10^{-7}.

Three types of algal assay experiments were carried out during this study: 1) nutrient limitation studies were performed for each Mill River sampling, 2) sewage additions or chemical equivalent nutrient additions were made in varying amounts to Mill River water and growth of Selenastrum capricornutum was monitored, and 3) additions of chemically treated sewage or treated chemical equivalent

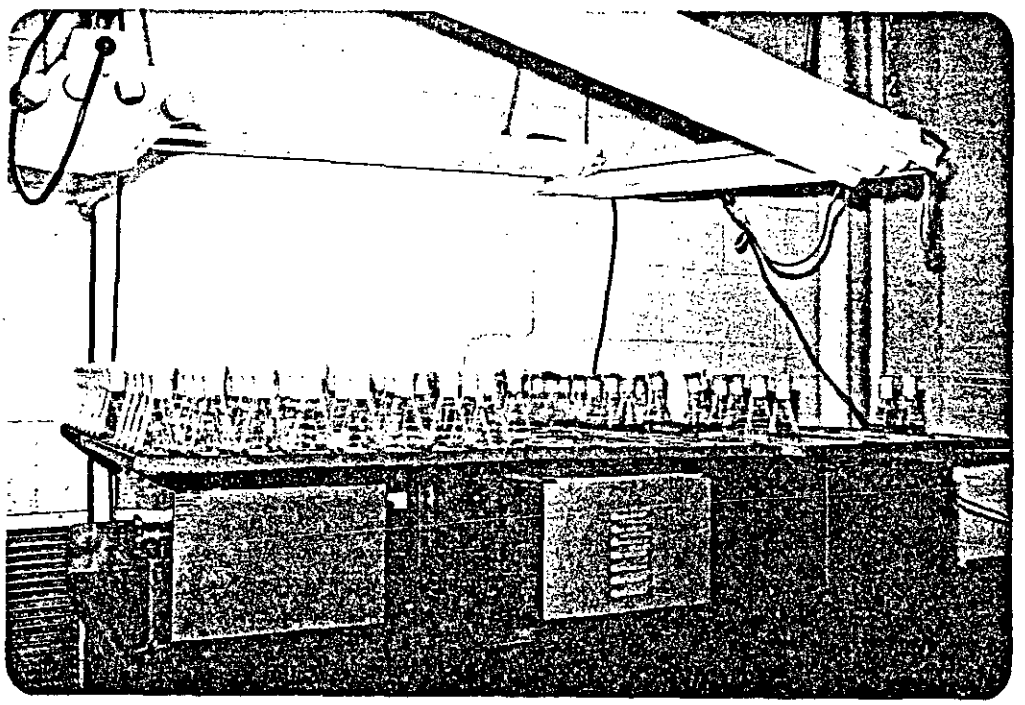


Figure 2-2. Laboratory set-up for algal assay experiment, showing shaker tables with culture flasks and fluorescent lighting.

nutrient additions in varying amounts to Mill River water were made and algal growth monitored. The chemical treatment removed phosphorus from the sample prior to algal assay.

Nutrient Limitation Studies

Nutrient limitation of the receiving water was determined by adding phosphorus, nitrogen, EDTA, and micronutrients, singly and in combination,to the samples. The following additions were made, to three replicates each of the Mill River.

Control (no addition)

Control + 0.05 mg/l P

Control + 1.0 mg/l N

Control + 0.05 mg/l P + 1.0 mg/l N

Control + 1.0 mg/l EDTA

Control + 0.05 mg/l P + 1.0 mg/l EDTA

Control + 1.0 mg/l N + 1.0 mg/l EDTA

Control + 0.05 mg/l P + 1.0 mg/l N + 1.0 mg/l EDTA

Control + micronutrients

The micronutrient addition was made from a stock solution which was a modification of the algal nutrient medium described by Miller, et al. (1978). Addition of the micronutrient spike resulted in the following concentrations of chemicals in the culture flasks.

Compound	Concentration	
$MgCl_2 \cdot 6H_2O$	12.164	mg/l
$CaCl_2 \cdot 2H_2O$	4.410	mg/l
$MgSO_4 \cdot 7H_2O$	14.700	mg/l
$NaHCO_3$	15.000	mg/l
H_3BO_3	185.520	ug/l
$MnCl_2 \cdot 4H_2O$	415.610	ug/l
$ZnCl_2$	3.271	ug/l
$CoCl_2 \cdot 6H_2O$	1.428	ug/l
$CuCl_2 \cdot 2H_2O$	0.012	ug/l
$Na_2MoO_4 \cdot 2H_2O$	7.260	ug/l
$FeCl_3 \cdot 6H_2O$	160.000	ug/l
$Na_2EDTA \cdot 2H_2O$	300.000	ug/l

Previous studies by researchers at the EPA Corvallis laboratory (Miller et al., 1978) have found that the maximum standing crop of *Selenastrum capricornutum*-Printz can be predicted from the inorganic nitrogen and phosphorus present. A ratio of approximately 11:1 inorganic nitrogen to orthophorus is ideal for Selenastrum growth, according to their findings. If the ratio or inorganic nitrogen to orthophosphorus (N:P ratio) is less than 11:1 there is an excess of nitrogen, and hence the water is phosphorus limited. Conversely, if the N:P ratio is greater than 11:1, there is an excess of phosphorus (relative to the nitrogen) and the water is nitrogen limited. For nitrogen limited waters, the MSC attained should be

$$\underset{(\text{mg/l dry wt.})}{\text{MSC}} = 38 \times \left(\underset{\text{mg/l}}{\text{Total Soluble Inorganic Nitrogen,}}\right) \pm 20\% \quad (2) .$$

For phosphorus limited waters the MSC attained should equal

$$\underset{(\text{mg/l dry wt.})}{\text{MSC}} = 430 \times \left(\underset{\text{mg/l}}{\text{Orthophosphorus,}}\right) \pm 20\% \quad (3) .$$

Chemical analyses can therefore be used to predict the MSC for the control and the samples containing chemical additions. This predicted value can be compared with actual MSC values determined by algal assay. EDTA is added to the samples to determine possible sample toxicity or micronutrient limitation. EDTA is known to chelate toxicants, especially heavy metals. Thus, if a toxicant is present in a water sample the addition of EDTA should increase algal growth relative to a sample without an EDTA addition. EDTA can also make micronutrients more available for algal growth in waters having low micronutrient levels. Increased algal growth in EDTA-spiked samples can therefore result from chelation of toxicants or increased micronutrient availability. A micronutrient spike was therefore additionally employed in the nutrient limitation studies to differentiate between these two possible modes of EDTA action.

Sewage Additions

Several experiments were conducted in which varying percentages of sewage were added to Mill River water. Maximum standing crop was determined by algal assay technique with no additional chemical spikes. The observed growth was compared to the predicted value based upon the nutrient content in the sewage and the Mill River dilution water. Micronutrient and EDTA spikes were added to

determine possible micronutrient limitation or toxicity in either the sewage or the dilution water.

A solution containing inorganic nitrogen and phosphorus nutrient constituents equivalent to that present in the sewage was prepared and added, in proportions corresponding to the sewage additions, to Mill river samples. Growth from the chemical equivalent additions was compared to the corresponding sewage additions. The chemical solution was prepared by dissolving in distilled water the following chemicals, in amounts equivalent to the concentrations present in the sewage. (The chemical content of the sewage was determined by the wet chemical techniques described previously.)

ortho-phosphorus--added as a K_2HPO_4, KH_2PO_4 buffer

ammonia-N--added as NH_4Cl

nitrite-N, nitrate-N--added as $NaNO_3$

micronutrients--added to the chemical solution such that the resulting concentrations were equal to those resulting from micronutrient stock solution additions to nutrient limitation study algal culture flasks, described previously.

The resulting solution was adjusted to the pH of the sewage. Alkalinity and hardness were adjusted to that of the sewage by adding $NaHCO_3$ and $CaCl_2 \cdot 2H_2O$ respectively.

Chemical Treatment

Secondary treated wastewater effluent and its chemical equivalent were treated with either lime or alum ($Al_2(SO_4)_3 \cdot 18H_2O$) to remove inorganic phosphorus . Varying percentages of the treated sewage or the sewage chemical equivalent solution were added to Mill River samples. Algal

growth resulting from these additions was observed.

Phosphorus was removed by chemical precipitation by adding a super-saturated solution of lime (CaO) as a slurry to each liter of sewage or its chemical equivalent. The lime slurry was added with rapid mixing using a variable speed stirring apparatus until pH 11.5 was attained. Rapid mixing was conducted at 100 rpm for 1 minute followed by flocculation at 30 rpm for 15 minutes. The supernatant was then filtered to remove precipitate.

A second method of phosphorus removal was also examined involving chemical precipitation by $Al_2(SO_4)_3 \cdot 18\ H_2O$ (alum). Alum was added to sewage or its chemical equivalent samples in a Al:P mole ratio of 1.5 : 1.0. The solutions were mixed for 30 seconds at 100 rpm, allowed to floculate by slow mixing at 20 rpm and then allowed to settle. Treatment was followed by filtration to remove precipitate.

CHAPTER 3

RESULTS

Chemical Determination

Colorimetric methods were used to determine orthophosphorus, total phosphorus, ammonia-N, nitrate-N, and nitrite-N. Absorbance readings at 885 nm, 885 nm, 635 nm, 540 nm, and 540 nm, respectively, were determined for known concentrations of chemical constituent. Standard absorbance versus concentration curves were constructed and used in future spectrophotometric determinations of unknown chemical constituent concentrations. Standard curves for these chemical constituents are shown in Figures 3-1 through 3-5.

Chemical Analysis of Mill River

The Mill River was sampled at four different times during the study. The sampling dates were June 16, 1981, June 24, 1981, July 16, 1981, and September 27, 1981. Ammonia-N, total organic nitrogen (TON), nitrate-N, nitrite-N, orthophosphorus, total phosphorus, alkalinity, hardness, and pH were determined at each sampling and nutrient limitation studies using algal assay were performed for the last three samplings. Chemical data are presented in Table 3-1.

The pH and hardness of Mill River water samples changed very little over the four times that it was sampled. The pH of the samples did change with autoclaving, but they were readjusted to the original pH values prior to algal assay. There was a

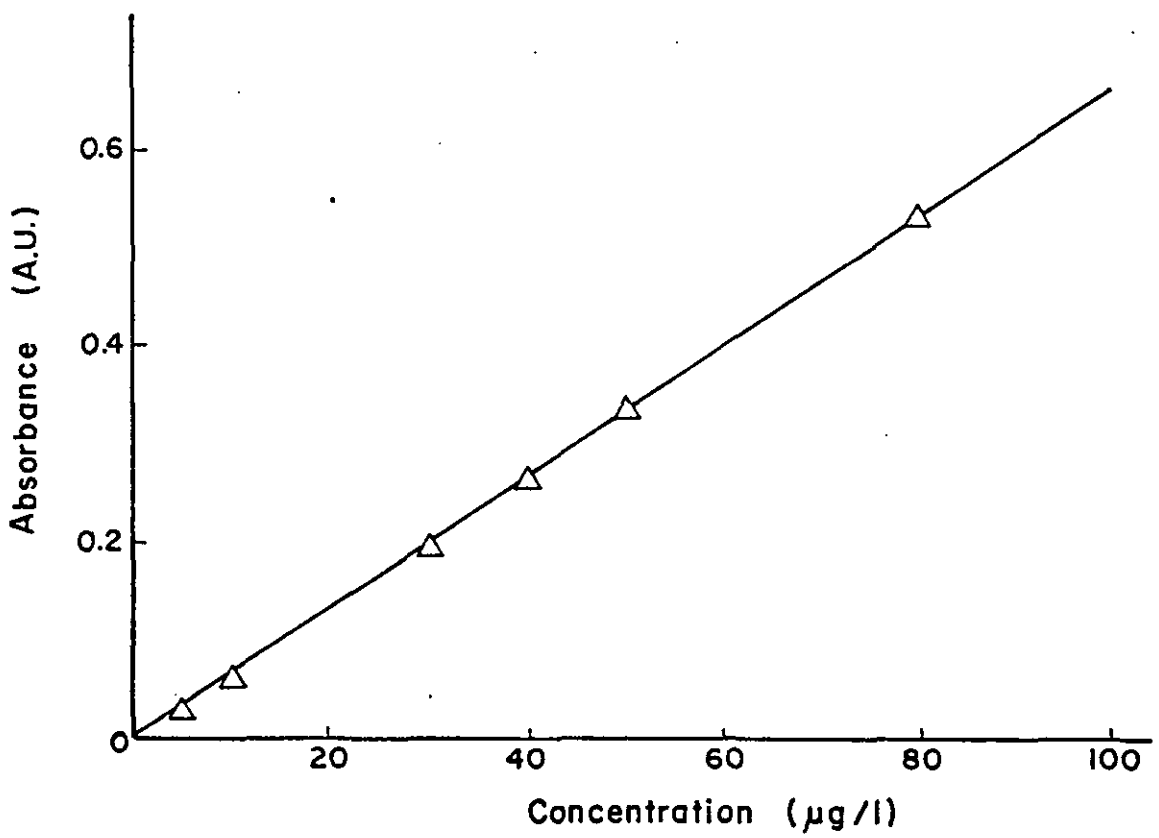


Figure 3-1. Standard curve for orthophosphorus (absorbance at 885 nm vs. concentration)

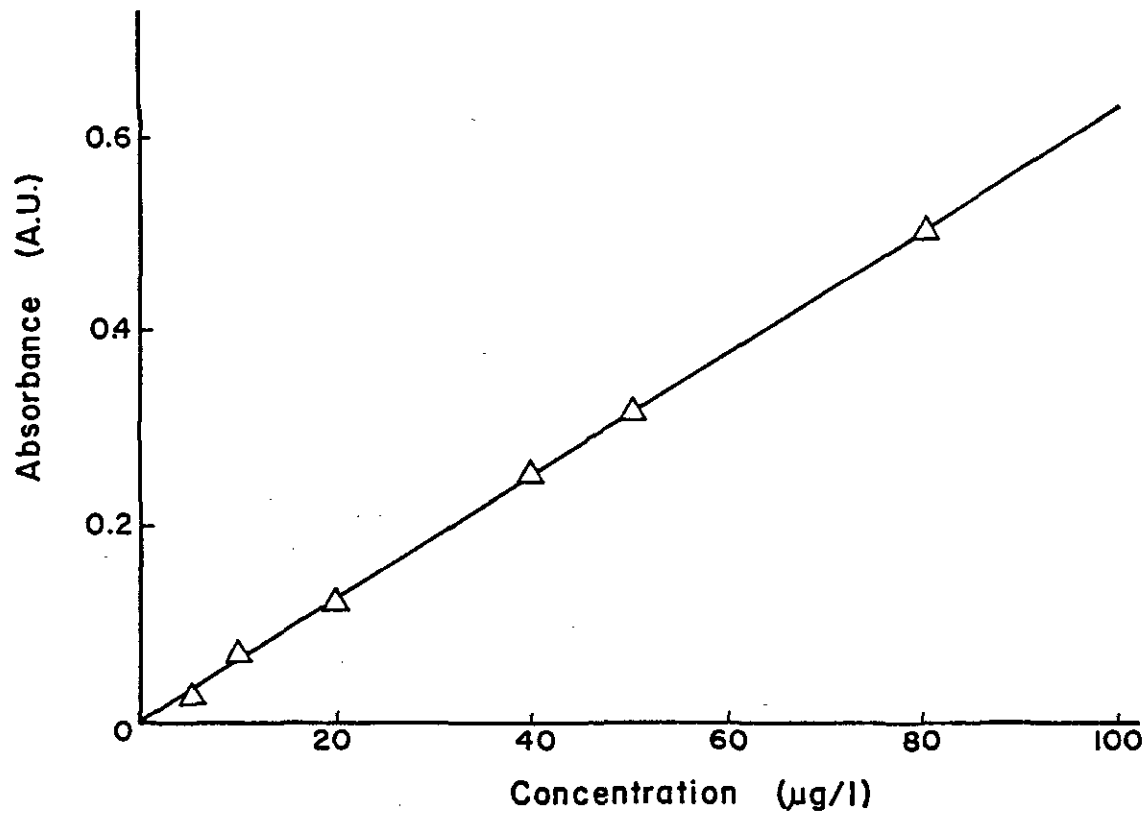


Figure 3-2. Standard curve for total phosphorus (absorbance at 885 nm vs. concentration)

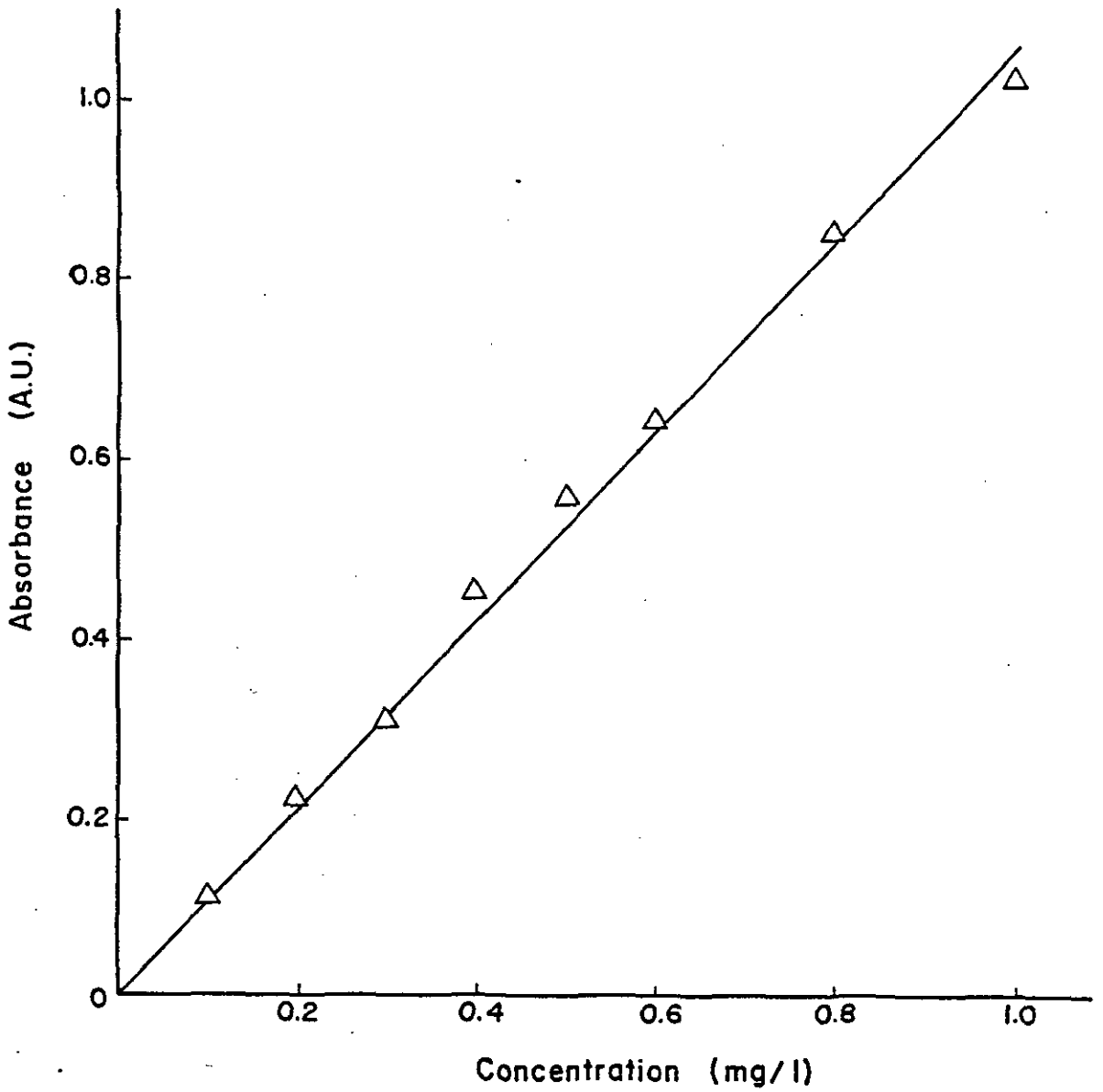


Figure 3-3. Standard curve for ammonia-N (absorbance at 635 nm vs. concentration)

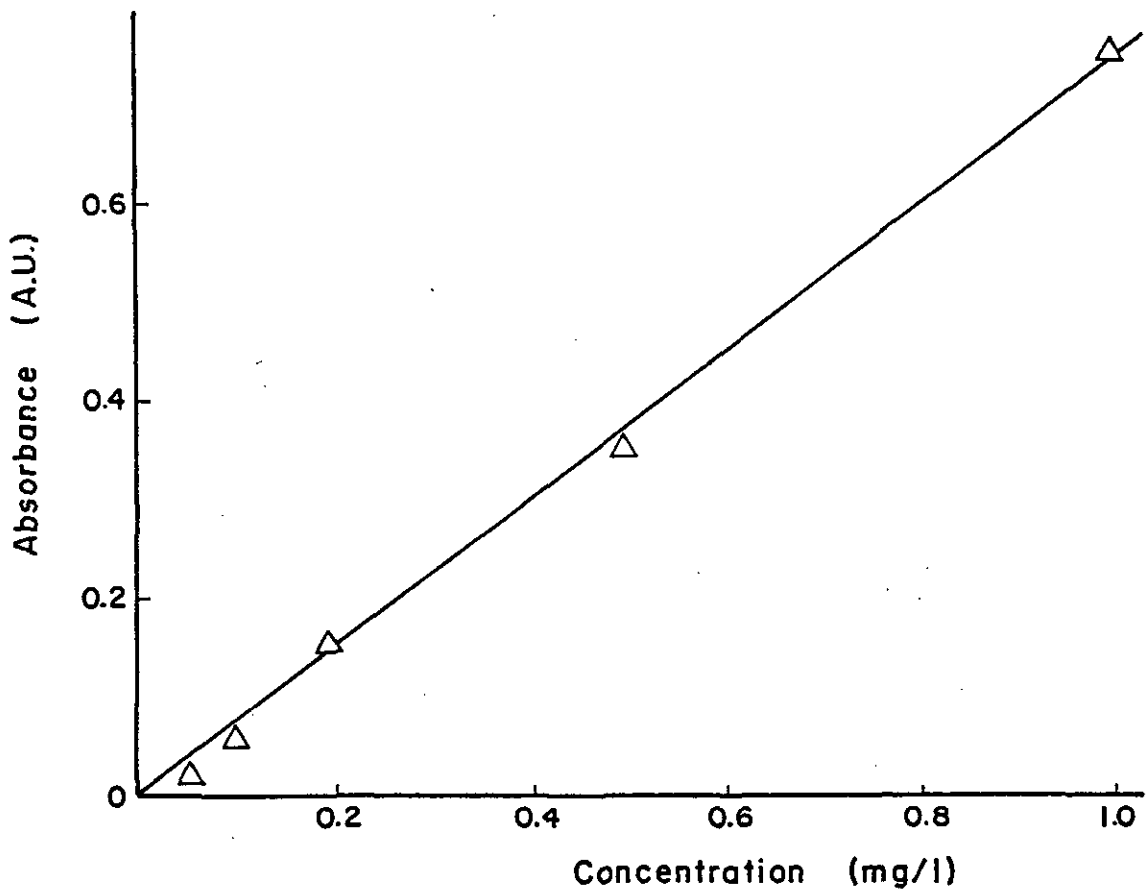


Figure 3-4. Standard curve for nitrate-N
(absorbance at 540 nm vs. concentration)

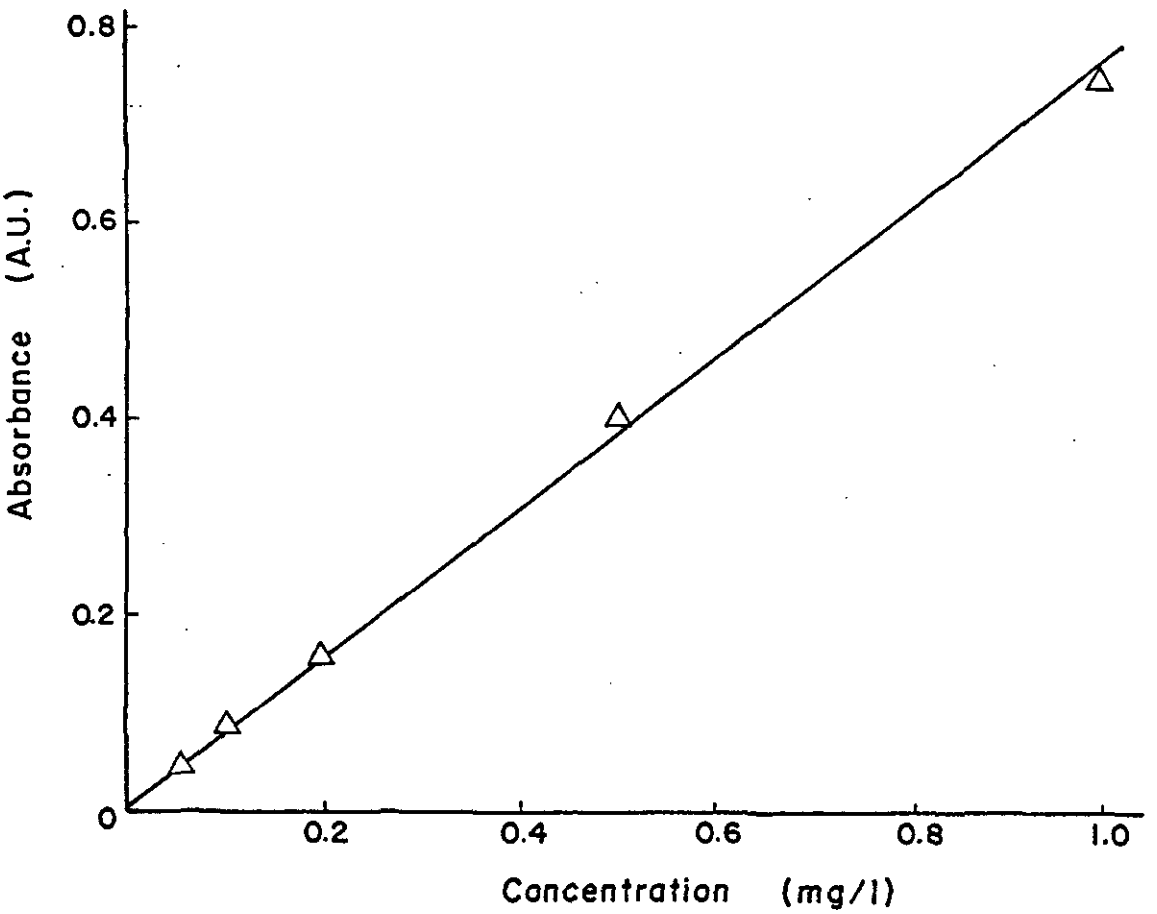


Figure 3-5. Standard curve for nitrite-N
(absorbance at 540 nm vs. concentration)

Table 3-1. Chemical analysis of Mill River water

Date	6/18/81		6/24/81		7/16/81		9/27/81	
treatment	NA[1]	A[2]	NA	A	NA	A	NA	A
pH	6.5		6.3		6.3		6.5	
alkalinity (mg/l as $CaCO_3$)	9.96	19.50	7.39	11.66	6.82	17.25	19.40	
hardness (mg/l as $CaCO_3$)	17.53	18.56	16.15	15.26	18.56	19.60	17.50	
ortho-P (mg/l)	0.005	0.026	0.070	0.056	0.006	0.012	0.002	0.003
total-P (mg/l)	0.034	0.049	0.036	0.070	0.010	0.025	0.021	0.029
nitrate-N (mg/l)			0.055	0.008	0.110	0.100	0.025	0.012
nitrite-N (mg/l)			0.004	0.102	0.000	0.000	0.000	0.000
ammonia-N (mg/l)	0.000	0.010	0.030	0.100	0.003	0.000	0.008	0.004
TSIN[3] (mg/l)			0.089	0.210	0.113	0.100	0.033	0.016
TON[4] (mg/l)	0.450	0.200	0.000	0.000	0.157	0.000	0.056	0.139
N:P ratio[5]			1.271	3.750	18.333	8.333	16.500	5.333

[1] NA = non-autoclaved

[2] A = autoclaved

[3] TSIN = total soluble inoganic nitrogen (NO_3-N + NO_2-N + NH_3-N)

[4] TON = total organic nitrogen

[5] N:P ratio = (TSIN/orthophosphorus)

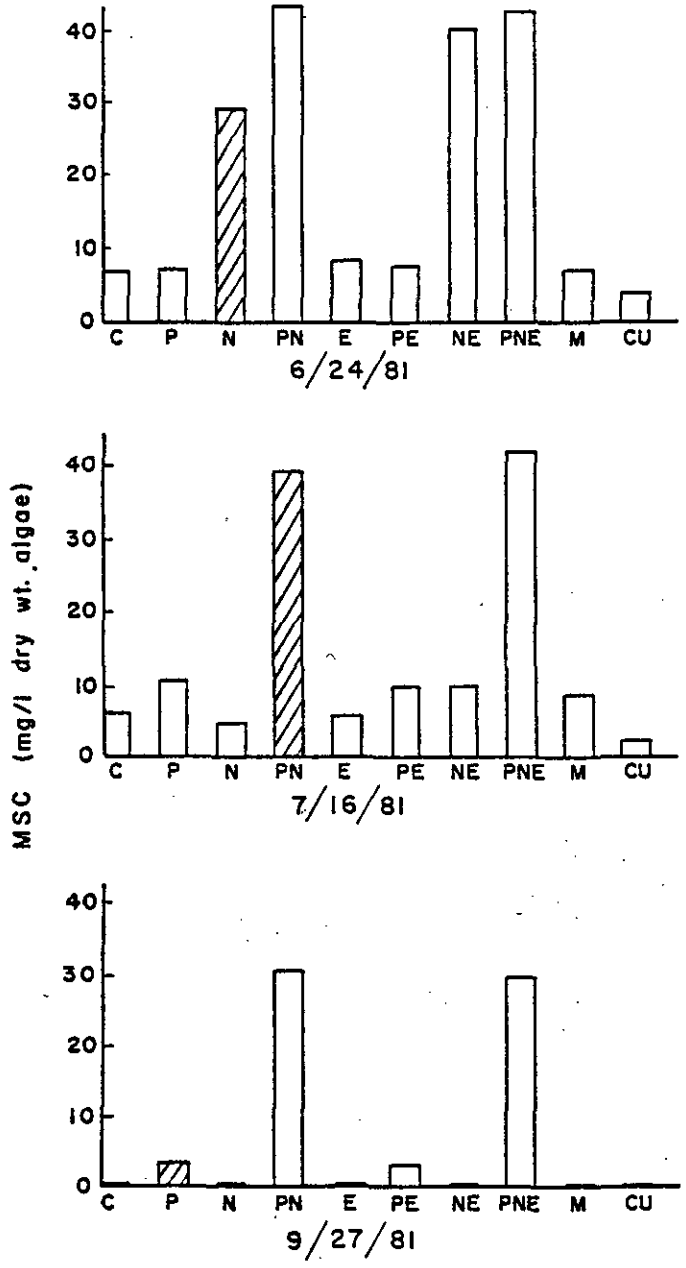
slightly greater range in the alkalinity of the samples over time sampled, but all would be considered low alkalinity values (EPA, 1979).

A considerable variation in phosphorus and nitrogen levels was observed over the sampling period. Total phosphorus values for autoclaved Mill River water ranged from 0.025 mg/l to 0.070 mg/l (mean = 0.043 mg/l, standard deviation = 0.021 mg/l) and ortho-phosphorus values for autoclaved river water ranged from 0.003 mg/l to 0.056 mg/l (mean = 0.024 mg/l, standard deviation = 0.023 mg/l). The total soluble inorganic nitrogen (TSIN), which is equal to the sum of nitrate-N, nitrite-N, and ammonia-N, ranged from 0.016 mg/l to 0.210 mg/l (mean = .109, standard deviation = 0.097 mg/l) for autoclaved Mill River water. The N:P ratio, which is equal to the ratio of TSIN to orthophosphorus, of autoclaved Mill River water remained under 9 in all cases, initially indicating that the Mill River was nitrogen-limited for algal growth (Miller, et al., 1978).

Nutrient Limitation Study of the Mill River

Nutrient limitation studies using Selenastrum capricornutum-Printz were performed on Mill River water samples collected on June 24, July 16, and September 27, 1981. Data on the maximum standing crop of the algae produced in samples containing Mill River water alone (control) or Mill river water plus chemical additions are presented in Figure 3-6. Hatch marks indicate the bar representing the growth response attributable to the limiting nutrient.

Figure 3-6. MSC (mg/l dry wt) Selenastrum capricornutum grown in Mill River plus chemical additions.[1]



1. C = control
P = + phosphorus spike
N = + nitrogen spike
PN = + phosphorus + nitrogen spikes
E = + EDTA spike
PE = + phosphorus + EDTA spikes
NE = + nitrogen + EDTA spikes
PNE = + phosphorus+nitrogen+EDTA spikes
M = + micronutrient spike
CU = control, unautoclaved

Chemical analyses of the water sampled June 24 exhibited a N:P ratio of 3.75 : 1.0, indicating nitrogen limitation. This was corroborated by the algal assay data, shown graphically in Figure 3-6. Nitrogen additions to sample aliquots resulted in an increase in the MSC while phosphorus additions had little effect on the MSC. Nitrogen plus phosphorus additions resulted in a larger increase in the MSC than the nitrogen spike alone. The EDTA plus nitrogen addition resulted in a somewhat larger MSC value than the nitrogen spike alone.

The maximum standing crop for a test water can be predicted based upon the inorganic nitrogen and orthophosphorus content using equations (2) and (3). Observed values were considered in good agreement with the predicted level if they fell within approximately ±20% of the predicted MSC. Comparison of predicted and observed algal yields for the June 24 sampling of the Mill River are shown in Table 3-2, and listed in Table A-1 of the Appendix. All but the nitrogen + EDTA data displayed good agreement between predicted and observed values. The observed yield of the control and nitrogen addition was just above the 20% value range, generally considered to indicate good agreement between observed and predicted values. However, the control plus nitrogen plus EDTA spike caused a much greater observed growth than that predicted from equation (3) and was attributed to experimental error.

If the observed MSC is divided by the TSIN level for nitrogen limited waters, or the orthophosphorus for phosphorus limited waters,

Table 3-2. Predicted and observed algal MSC values (mg/l dry wt) for Mill River sampled June 24, July 16, and September 27.

	June 24, 1981		July 16, 1981		September 27, 1981	
	P[1] mg/l dry wt.	O[2] mg/l dry wt.	P mg/l dry wt.	O mg/l dry wt.	P mg/l dry wt.	O mg/l dry wt.
MRW[3]	7.98	6.42	3.67	6.14	0.59	0.32
+ P	7.98	6.81	3.67	10.70	0.59	2.91
+ N	23.94	29.08	5.16	4.45	1.28	0.21
+ EDTA	7.98	7.82	3.67	5.50	0.59	0.20
+ P + N	45.58	42.66	26.66	38.90	22.78	29.79
+ P + EDTA	7.98	7.62	3.67	9.49	0.59	2.71
+ N + EDTA	23.94	40.02	5.16	9.49	1.28	0.22
+ P + N + EDTA	45.58	42.23	26.66	41.25	22.78	29.29
+ micronutrients	7.98	7.12	3.67	8.65	0.59	0.27
non-autoclaved	3.36	3.99	2.58	2.26	0.59	0.21

[1] P = predicted MSC, calculated using

MSC (mg/l dry wt.) = 38 X TSIN (mg/l) (2) for N - limited water

MSC (mg/l dry wt.) = 430 X ortho-P (mg/l) (3) for P-limited water

[2] O = observed MSC

[3] MRW = Mill River water

a growth factor is obtained which reflects the level of nutrient bioavailability. This factor can also be used to compare observed results with predicted values. For nitrogen limited waters dividing the observed MSC value by the TSIN concentration usually yields a growth factor between 30.4 and 45.6 . In waters phosphorus limited for algal growth dividing observed MSC values by the orthophosphorus concentration usually results in a growth factor between 344 and 516. Growth factors for nutrient limitation studies of the Mill River are presented in Table 3-3. Again, there is agreement of the observed and predicted data for the June sampling for all of the chemical treatments with the exception of nitrogen and nitrogen plus EDTA.

Chemical analyses of water sampled on July 16 from the Mill River showed a N:P ratio of 8.33 : 1.0 indicating the water was nitrogen limited, based on findings of Miller et al (1978). Chiaudani and Vighi (1976) have found waters with N:P ratios as low as 5.0 : 1.0 to need additions of both nitrogen and phosphorus for algal growth. The observed MSC of algae resulting from various spike additions indicated that the water was indeed co-limited. Maximum standing crop data from this algal assay are reported in the Appendix in Table A-4. Figure 3-6 shows that the algal response to the control, control plus phosphorus, control plus EDTA, and control plus nitrogen were all similar. Substantial increase in the MSC was only observed with the control plus nitrogen plus phosphorus, and the control plus nitrogen plus phosphorus plus EDTA samples,

Table 3-3. Mill River nutrient limitation study observed Growth Factors.[1]

	N:P ratio			Growth Factor					
				June 24, 1981		July 16, 1981		Sept. 27, 1981	
	6/24	7/16	9/27	P limited	N limited	P limited	N limited	P limited	N limited
MRW[2]	3.75	8.33	4.00		30.57	511.67			20.64
MRW + EDTA					37.23	458.33			12.84
MRW + P	1.98	1.61	0.23		32.43		110.98		187.74
MRW + P + EDTA					36.57		98.34		174.84
MRW + N	21.61	84.33	337.33	519.29		370.83		70.00	
MRW + N + EDTA				714.64		790.83		73.00	
MRW + P + N	11.42	17.74	19.09	402.45	35.26	627.42		562.08	
MRW+P+N+EDTA				398.40	34.90	665.32		552.64	

[1]Observed growth factor for N-limited waters = MSC (mg/l dry wt.) ÷ (TSIN),
for P-limited waters = MSC (mg/l dry wt.) ÷ (orthophosphorus).
Predicted growth factors for N-limited waters lie between 30.4 and 45.6,
for P-limited waters lie between 344 and 516 .

[2]MRW = Mill River Water

indicating that the sample may have been co-limited by nitrogen and phosphorus for algal growth.

The analysis of the water sampled on September 27 revealed extremely low nutrient levels, indicated by both orthophosphorus and total soluble inorganic nitrogen. Ammonia-N and nitrate-N concentrations were below detectable levels. Chemical analyses indicated nitrogen limitation, but the algal growth response shown graphically in Figure 3-6 and the observed MSC values presented in Tables 3-2 and A-10 indicated phosphorus limitation. The data suggests likelihood of some analytical error during chemical analyses of inorganic nutrients.

Effect of EDTA and Micronutrient Additions

T-tests were performed on data obtained from the nutrient limitation algal assays to determine if the algal response in cultures containing EDTA or micronutrient additions were significantly different from those without additions. The MSC for each nutrient addition was compared to the MSC value obtained in the corresponding algal culture containing EDTA or micronutrient addition. The two MSC values were then subjected to a t-test to determine whether they were both taken from the same sample data population. A statistical comparison was performed using the equations described by Daniel (1978)

In order to test the hypothesis that the two MSC values were from the same data population, and not statistically different from each other (H_o: $x_1=x_2$) the standard deviations (s_1 and s_2)

of the two MSC values were first compared with each other using an f test, where

$$f = \frac{(s_1)^2}{(s_2)^2} \quad , \tag{4}$$

to determine if s_1 and s_2 were homoskidastic at the 99 % confidence level. If the two standard deviations were homoskidastic a pooled variance (s_p^2) was then calculated using the equation:

$$s_p^2 = \frac{s_1^2 (n_1 - 1) + s_2^2 (n_2 - 1)}{(n_1 + n_2 \div 2)} \quad . \tag{5}$$

A t-statistic was then calculated from the two MSC values and their corresponding standard deviations, using the equation:

$$t = \frac{x_1 - x_2}{s_p \left(\frac{1}{n_1} + \frac{1}{n_2} \right)^{\frac{1}{2}}} \quad . \tag{6}$$

The calculated t value was then compared to values taken from a student t distribution the the 99 % confidence level for $n_1 + n_2 - 2$ degrees of freedom. If the calculated t value was outside the tabulated t value at the 99 % confidence level the hypothesis was rejected and the two MSC values were considered statistically different from each other at the 99 % confidence level.

If the f test indicated that the standard deviations of the two MSC values were not homoskidastic, the variances cannot be pooled. The t statistic was then calculated using:

$$t = \frac{(x_1 - x_2)}{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2} \right)^{\frac{1}{2}}} \tag{7}$$

with

$$\text{degrees of freedon} = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\left(\frac{(s_1^2/n_1)^2}{n_1} + \frac{(s_2^2/n_2)^2}{n_2}\right)} \quad . \qquad (8)$$

The results of these analyses are presented in Table 3-4 and Appendix Tables A-2, A-5, and A-11. In 7 out of 15 pairs of algal treatments with and without EDTA or micronutrients the growth response was significantly different at the 99 % confidence level. In all but one of these 7 data sets, the algal cultures containing EDTA or micronutrient additions achieved greater MSC values than in those without these additions.

While the t test results did indicate a statistically significant effect resulting from EDTA or micronutrient additions in slightly less than half of the cases, the numerical MSC values were very close in all but one instance. This observed difference may therefore have been attributable to minor dilution error or natural variability in algal growth response. This hypothesis is supported by previous studies by Miller (1973 and 1976) in which observed MSC values were much less than predicted values. A consistant and marked increase in algal growth, therefore, is usually expected in samples containing toxicants or limited by micronutrients when EDTA or micronutrient spikes, in the latter condition, are added. The Mill River data reported here, then, does not clearly indicate either the presence of toxicants or micronutrient limitation.

Table 3-4. Effect of EDTA and Micronutrient additions on Selenastrum capricornutum grown in Mill River water.

	MSC(mg/l dry wt.)			6/24/81		7/16/81		9/27/81		H_o: $x_1 = x_2$		
	6/24	7/16	9/27	t_c[2]	$t_{.995}$[3]	t_c	$t_{.995}$	t_c	$t_{.995}$	6/24	7/16	9/27
MRW[1]	6.42	6.14	0.32	-17.89	3.25	+1.28	3.11	+5.27	2.92	R[4]	A[5]	R
MRW + EDTA	7.82	5.50	0.20									
MRW + P	6.81	10.70	2.91	-8.95	3.25	+0.41	3.11	+1.36	3.17	R	A	A
MRW + P + EDTA	7.61	9.49	2.71									
MRW + N	29.08	4.45	0.21	-31.49	2.92	-3.99	2.92	-1.06	2.92	R	R	A
MRW + N + EDTA	40.02	9.49	0.22									
MRW + P + N	42.66	38.90	29.79	+ 0.97	3.25	-5.51	2.92	+0.80	2.92	A	R	A
MRW+P+N+EDTA	42.23	41.25	29.29									
MRW	6.42	6.14	0.32	-12.43	3.25	-2.13	2.92	+2.23	2.92	R	A	A
MRW + micronu-trient	7.12	8.65	0.27									

1. MRW = Mill River Water
2. $t_c = t_{calculated}$ using equation (6) or (7).
3. $t_{.995}$ varies depending upon sample number, standard deviation, and degrees of freedom.
4. R = reject hypothesis $x_1 = x_2$ (MSC values are statistically different), when $t_{.995}$ is less than $|t_c|$.
5. A = accept hypothesis $x_1 = x_2$ (MSC values are not statistically different), when $t_{.995}$ is greater than $|t_c|$.

Sewage Additions to Mill River Water Sampled June 24, 1981

Secondary treated wastewater effluent was added to Mill River aliquots in 1, 5, 10, 30, 50, 70, and 100 % additions. Chemical analyses of the wastewater effluent sampled June 24 and on the two subsequent sampling dates are presented in Table 3-5. Both chemical analyses and algal assays indicated that algal growth was nitrogen limited in all cases. Maximum standing crop data for Selenastrum capricornutum are shown in Table 3-6 and in Appendix Table A-1. The observed yield was much greater than the range of predicted yields based on TSIN, an all cases except the 1 % addition. This indicated that the algae may have been utilizing organic nitrogen present in the sewage resulting in larger algal growth yields than that predicted by TSIN alone. However, the observed MSC values were greater than the predicted values which incorporated the organic nitrogen component of the wastewater effluent as well.

Figure 3-7 shows the algal MSC (mg/l dry wt.) versus the percent sewage addition and the TSIN(mg/l). A linear regression is plotted for observed yield. The equation of this line is

$$\text{MSC (mg/l dry wt.)} = 54.49 \times \text{TSIN (mg/l)} - 0.38 \qquad (9)$$

with an r value of 0.9981. This value is considerably higher than the predicted yield equation of

$$\text{MSC (mg/l dry wt.)} = 38.0 \times \text{TSIN (mg/l)} \pm 20\ \% \qquad (2)$$

Thus the observed values are significantly higher than the predicted values at all levels other than the 1 % sewage addition.

Table 3-5. Chemical analysis of Amherst wastewater treatment plant composite samples.

Date	June 24, 1981	July 16, 1981	Sept. 27, 1981
pH	7.0	6.8	7.0
alkalinity (mg/l as $CaCO_3$)	51.13	35.96	61.90
hardness (mg/l as $CaCO_3$)	70.10	84.54	84.50
orthophosphorus (mg/l)	2.66	3.04	4.07
total phosphorus (mg/l)	3.56	3.24	4.72
organic-P[1] (percent)	25.28	6.17	13.77
nitrate-N (mg/l)	4.36	11.50	10.50
nitrite-N (mg/l)	0.33	0.25	0.23
ammonia-N (mg/l)	0.91	1.16	4.40
TSIN[2] (mg/l)	5.60	12.91	15.13
TON[3] (mg/l)	0.28	0.57	0.81
organic-N[4] (percent)	4.76	4.22	5.08
N : P ratio [5]	2.11	4.25	3.72

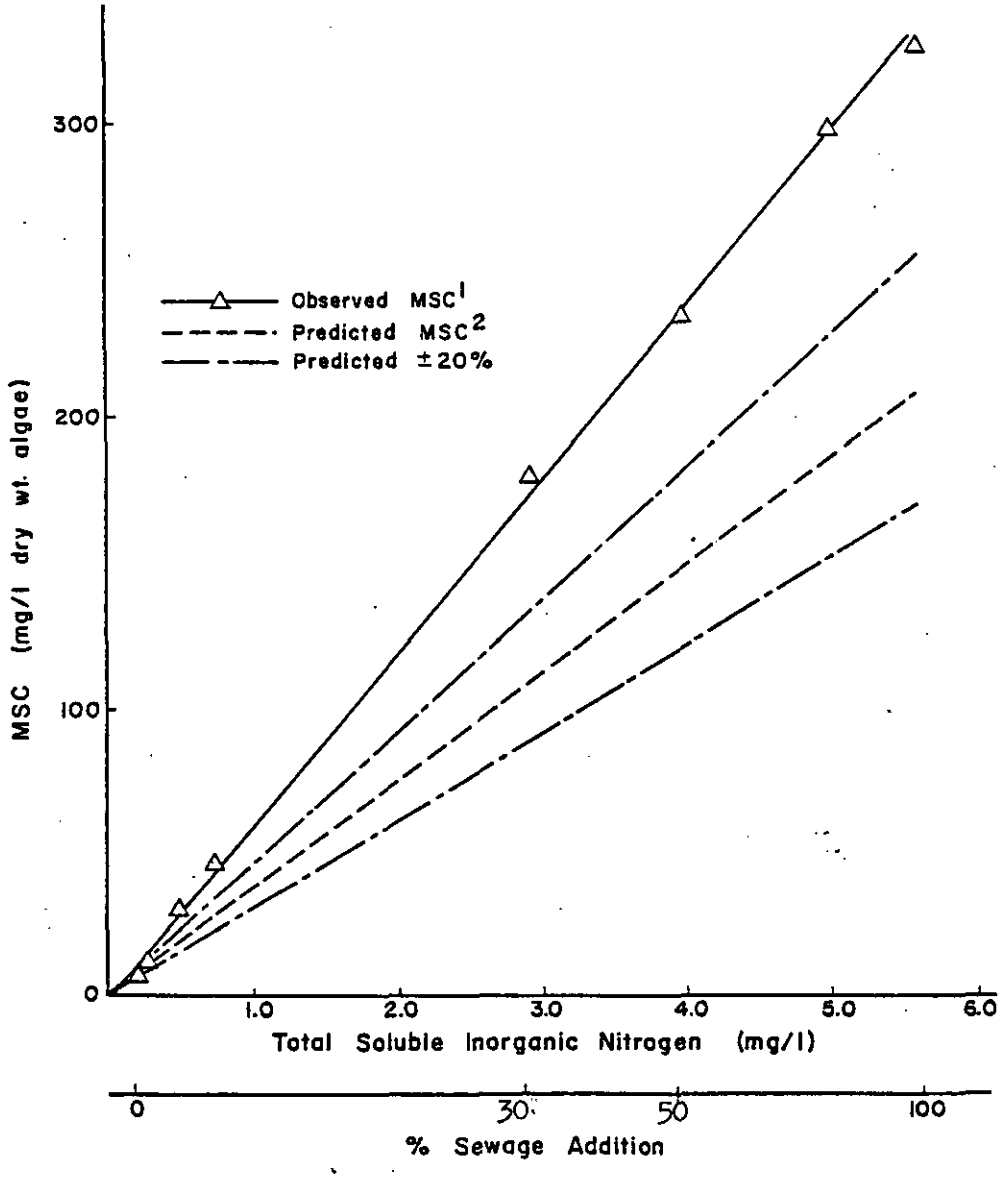
1. Organic-P = (1-ortho-P/total-P) X 100
2. TSIN = total soluble inorganic nitrogen = NO_2-N + NO_3-N + NH_3-N.
3. TON = total organic nitrogen
4. organic-N = (TON/(TON + TSIN)) X 100
5. N:P ratio = TSIN/ortho-P

Table 3-6. Predicted and observed MSC (mg/l dry wt.) of Selenastrum capricornutum grown in Mill River water sampled June 24, 1981 plus sewage additions.

	Predicted MSC[2] (mg/l dry wt.)	Predicted MSC[3] (mg/l dry wt.)	Observed MSC (mg/l dry wt.)
MRW[1]	7.98 (6.38- 9.56)[4]	7.98 (6.38- 9.56)[4]	6.42
+ 1 % Sewage	10.03 (8.02- 12.03)	10.13 (8.10- 12.16)	10.24
+ 1 % Sewage + EDTA			11.11
+ 5 % Sewage	18.22 (14.57- 21.86)	18.74 (14.99- 22.49)	27.00
+ 5 % Sewage + EDTA			25.93
+ 10 % Sewage	28.45 (22.76- 34.14)	29.50 (23.60- 35.40)	44.90
+ 10 % Sewage + EDTA			43.97
+ 30 % Sewage	69.39 (55.51- 83.27)	72.54 (58.03- 87.05)	122.47
+ 30 % Sewage + EDTA			122.32
+ 50 % Sewage	110.33 (88.27-132.40)	115.58 (92.46-138.69)	180.54
+ 50 % Sewage + EDTA			182.85
+ 70 % Sewage	151.27 (121.02-181.53)	158.62 (126.89-190.34)	233.71
+ 70 % Sewage + EDTA			245.54
+100 % Sewage	212.69 (170.15-255.22)	223.17 (178.54-267.81)	326.36
+100 % Sewage + EDTA			323.72

1. MRW = Mill River Water
2. Predicted yield = 38 X (TSIN) $\pm$ 20 % (2)
3. Predicted yield = 38 X (TSIN + TON) $\pm$ 20 %
4. Values in parentheses represent acceptable range ($\pm$ 20%) of predicted values.

Figure 3-7. Observed and predicted MSC (mg/l dry wt.) Selenastrum capricornutum grown in Mill River water with sewage additions, June 24, 1981.



1. Observed MSC (mg/l dry wt.) = 54.49 X TSIN (mg/l)-0.38 (9)
2. Predicted MSC (mg/l dry wt.) = 38.0 X TSIN (mg/l) ± 20 % (2)

The t statistic was computed by comparing MSC values at each level of sewage additon with the MSC value for the same sewage level with an EDTA addition. Equations (4) through (8) were used, and the calculated t statistic was compared with tabulated t values at the 99% confidence level with the appropriate degrees of freedom. These calculations are shown in Table A-3, and are summarized in Table 3-7. The difference in MSC values for a percentage sewage addition and the corresponding percentage addition plus EDTA was statistically significant at only the 1 % and 70 % levels. In both cases, however, the actual difference in the two MSC values was less than 10 percent. As these values are so close, and the EDTA did not significantly affect algal growth in the remaining 5 of the 7 additions, it is unlikely that toxicants were present or that there was micronutrient limitation in either the added sewage or the Mill River water diluent.

Table 3-7. Effect of EDTA additions on Selenastrum capricornutum grown in Mill River water with sewage additions, June 24, 1981.

Mill River water plus	MSC (mg/l dry. wt)	t_c [1]	$t_{.995}$ [2]	H_o: $x_1 = x_2$
1 % sewage	10.24	- 3.88	3.17	Reject [3]
1 % sewage,EDTA	11.11			
5 % sewage	27.00	+ 2.57	3.11	Accept [4]
5 % sewage,EDTA	25.93			
10 % sewage	44.90	+ 1.33	2.92	Accept
10 % sewage,EDTA	43.97			
30 % sewage	122.47	+ 0.14	2.92	Accept
30 % sewage,EDTA	122.32			
50 % sewage	180.54	- 1.83	2.92	Accept
50 % sewage,EDTA	182.54			
70 % sewage	233.71	- 4.43	2.92	Reject
70 % sewage,EDTA	245,54			
100 % sewage	326.36	+00.10	2.92	Accept
100 % sewage,EDTA	323.72			

1. $t_c = t_{calculated}$ using equation (6) or (7).
2. $t_{.995}$ varies depending upon calculated degrees of freedom.
3. reject hypothesis $x_1=x_2$ (MSC values are statistically different) when $t_{.995}$ is less than $|t_c|$.
4. accept hypothesis $x_1=x_2$ (MSC values are not statistically different) when $t_{.995}$ is greater than $|t_c|$.

Sewage Additions to Mill River Water sampled July 16, 1981

Secondary treated wastewater effluent sampled July 16 was added to Mill River water for algal assay. Chemical analyses of the sewage are given in Table 3-5. Observed maximum standing crop values for Selenastrum capricornutum are given in Tables 3-8 and A-6. Observed growth was within the range of predicted yields in all cases. Theoretical yields were based on TSIN values in the sewage and in the Mill River water. Growth was nitrogen limited at all levels of sewage addition. Figure 3-8 shows algal yield (mg/l dry wt.) plotted against percent sewage addition and TSIN (mg/l). A linear regression line was constructed from the observed data and is defined by the equation:

$$MSC\ (mg/l\ dry\ wt.) = 30.54 \times (TSIN) + 11.54 . \qquad (10)$$

The regression line had an r factor equal to 0.9956.

EDTA and micronutrient additions were also evaluated in this assay. Statistical analysis was performed on resulting algal yields to determine if either micronutrient or EDTA additions affected algal growth. The t statistic was calculated and compared to tabulated values at the 99 % confidence level with appropriate degrees of freedom. These calculations are listed in Table A-6 and are summarized in Table 3-9. Algal yields were not significantly affected by addition of micronutrient or EDTA at the 5 % or 20 % sewage addition level. At the 10 % sewage addition level the cultures containing EDTA had yields significantly lower

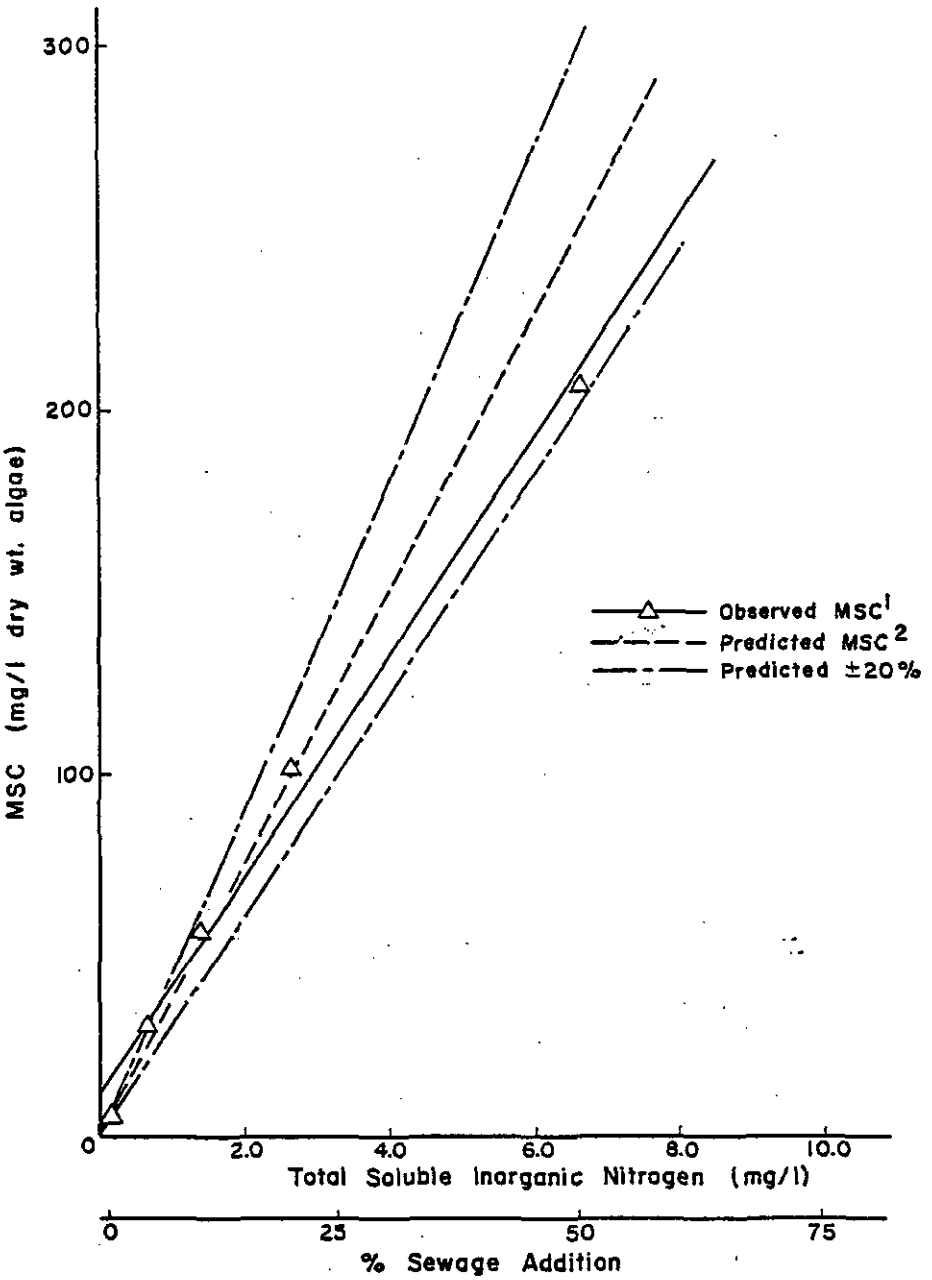
than the cultures with no EDTA addition. Only at the 50 % sewage addition level did cultures with EDTA and micronutrient additions achieve yields significantly greater than the algal cultures grown without these spike additions. Thus, cultures grown with EDTA or micronutrient additions achieved significantly greater yields at only one of the four sewage addition levels, and in both cases the difference in the compared MSC values was less than 11%. It is therefore possible that the higher observed algal yields in cultures containing micronutrient or EDTA additions were due to experimental variability and not caused by micronutrient limitation in either the Mill River water or the wastewater effluent.

Table 3-8. Predicted and observed MSC (mg/l dry wt.) of Selenastrum capricornutum grown in Mill River water sampled July 16, 1981, plus sewage additions.

Mill River water plus	Predicted MSC[1] (mg/l dry wt.)	Observed MSC (mg/l dry wt.)
no addition	3.67 (2.94- 4.40)[2]	6.14
+ 5 % sewage	28.22 (22.57- 33.86)	32.97
+ 5 % sewage, EDTA		33.22
+ 5 % sewage, micronutrients		31.99
+ 10 % sewage	52.65 (42.11- 63.18)	58.42
+ 10 % sewage, EDTA		54.45
+ 10 % sewage, micronutrients		57.42
+ 20 % sewage	101.52(81.22-121.82)	102.45
+ 20% sewage, EDTA		104.25
+ 20 % sewage, micronutrients		104.38
+ 50 % sewage	248.13 (198.50-297.76)	205.70
+ 50 % sewage, EDTA		227.78
+ 50 % sewage, micronutrients		222.22

1. Predicted yield = 38 X TSIN (mg/l) ± 20 %. (2)
2. Values in parentheses represent acceptable range (± 20 %) of predicted values.

Figure 3-8. Observed and predicted MSC (mg/l dry wt.) Selenastrum capricornutum grown in Mill River water with sewage additions, sampled July 16, 1981.



1. Observed MSC (mg/l dry wt.) = 30.54 X TSIN + 11.54 (10)
2. Predicted MSC (mg/l dry wt.) = 38.0 X TSIN ± 20 % (2)

Table 3-9. Effect of EDTA and micronutrient additons on Selenastrum capricornutum grown in Mill River water with sewage additions, both sampled July 16, 1981.

Mill River water plus	MSC (mg/l dry wt)	t_c [1]	$t_{.995}$	$H_o: x_1 = x_2$
5% sewage 5% sewage, EDTA	32.97 33.22	- 0.46	2.92	Accept[2]
5% sewage 5% sewage, micronutrients	32.97 31.99	+ 0. 66	2.92	Accept
10% sewage 10% sewage, EDTA	58.41 54.45	+ 3.51	2.92	Reject[3]
10% sewage 10% sewage, micronutrients	58.41 57.42	+ 1.73	2.92	Accept
20% sewage 20% sewage, EDTA	102.45 104.25	- 1.11	2.92	Accept
20% sewage 20% sewage, micronutrients	102.45 104.38	- 1.67	2.92	Accept
50% sewage 50% sewage, EDTA	205.70 227.78	- 5.65	2.92	Reject
50% sewage 50% sewage, micronutrients	205.70 222.22	- 4.10	2.92	Reject

1. $t_c = t_{calculated}$ using equation (6) or (7).

2. accept hypothesis $x_1 = x_2$ (MSC values are not statistically different) when $t_{.995}$ is greater than $|t_c|$.

3. reject hypothes s $x_1 = x_2$ (MSC values are statistically different) when $t_{.995}$ is less than $|t_c|$.

Chemical Equivalent Solution addition to Mill River water sampled July 16, 1981

A chemical equivalent solution (CES) of the sewage was prepared as described in the Methods section and added to Mill River water sampled July 16, 1981. Due to error in initial chemical analysis of the sewage, the equivalent solution contained less than half the nitrate concentration present in the actual sewage. Orthophosphorus levels were equal in the sewage and the chemical equivalent solution. Thus, a 5 % addition (by volume) contained a TSIN concentration equal to that of a 2.1 % addition of sewage. Percent addition values shown in Table 3-10 and Figure 3-9 have been corrected for this error and are expressed as equivalent percent additions of TSIN. The observed yields were slightly higher than the predicted MSC values at all levels of addition other than 50 % (by volume) chemical equivalent solution addition.

The observed MSC values for the varying percentage additions to Mill River water plotted against TSIN (mg/l) are shown in Figure 3-9. The least squares fit equation passing through these data points was calculated to be:

$$\text{MSC (mg/l dry wt.)} = 30.48 \times \text{TSIN (mg/l)} + 11.94 \qquad (11)$$

with an r value of 0.9783. Observed algal yields (mg/l dry wt.) for both sewage and chemical equivalent solution additions are plotted versus TSIN (mg/l) in Figure 3-10. This figure illustrates that there is close agreement between the algal stimulatory potential in sewage and the chemical equivalent solution.

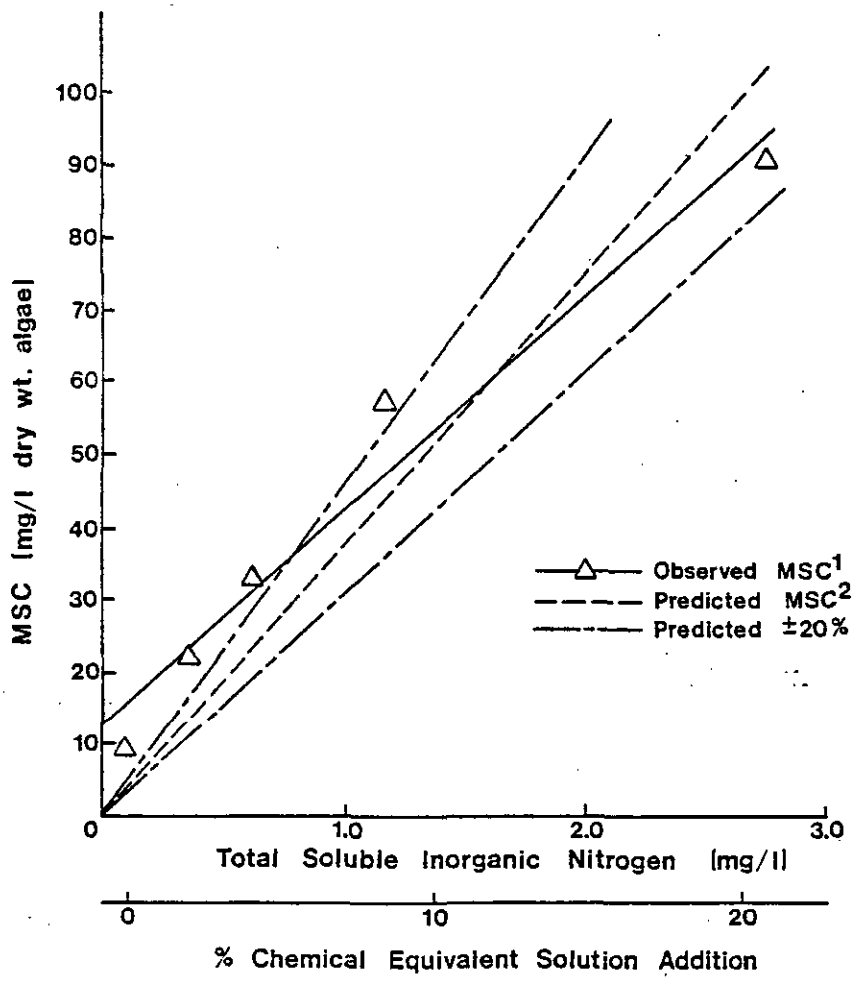
The observed MSC values at each percentage level of chemical equivalent solution addition for algal cultures with and without EDTA and micronutrient additions were compared using equations (4) through (8). The results are presented in Table A-8 in the Appendix, and summarized in Table 3-11. At the 99% confidence level, statistical tests indicated that there were no significant effects on MSC yields attributable to either EDTA or micronutrient additions.

Table 3-10. Predicted and observed MSC (mg/l dry wt.) of Selenastrum capricornutum grown in Mill River water sampled July 16, 1981 plus chemical equivalent solution additions.

Mill River plus addition (by volume)	= TSIN[2] sewage	Predicted MSC[3] (mg/l dry wt.)	Observed MSC (mg/l dry wt)
no addition		3.67 (2.94- 4.40)	8.90
+ 5% CES[1]			20.70
+ 5% CES, EDTA	2.1%	13.84 (11.07- 16.61)	21.84
+ 5% CES, micronutrient			20.49
+10% CES			33.29
+10% CES, EDTA	4.2%	23.89 (19.12- 28.69)	38.87
+10% CES, micronutrient			32.63
+20% CES			58.04
+20% CES, EDTA	8.4%	44.02 (35.22- 52.82)	67.27
+20% CES, micronutrient			56.43
+50% CES			91.12
+50% CES, EDTA	20.9%	104.38 (83.50-125.30)	105.33
+50% CES, micronutrient			104.38

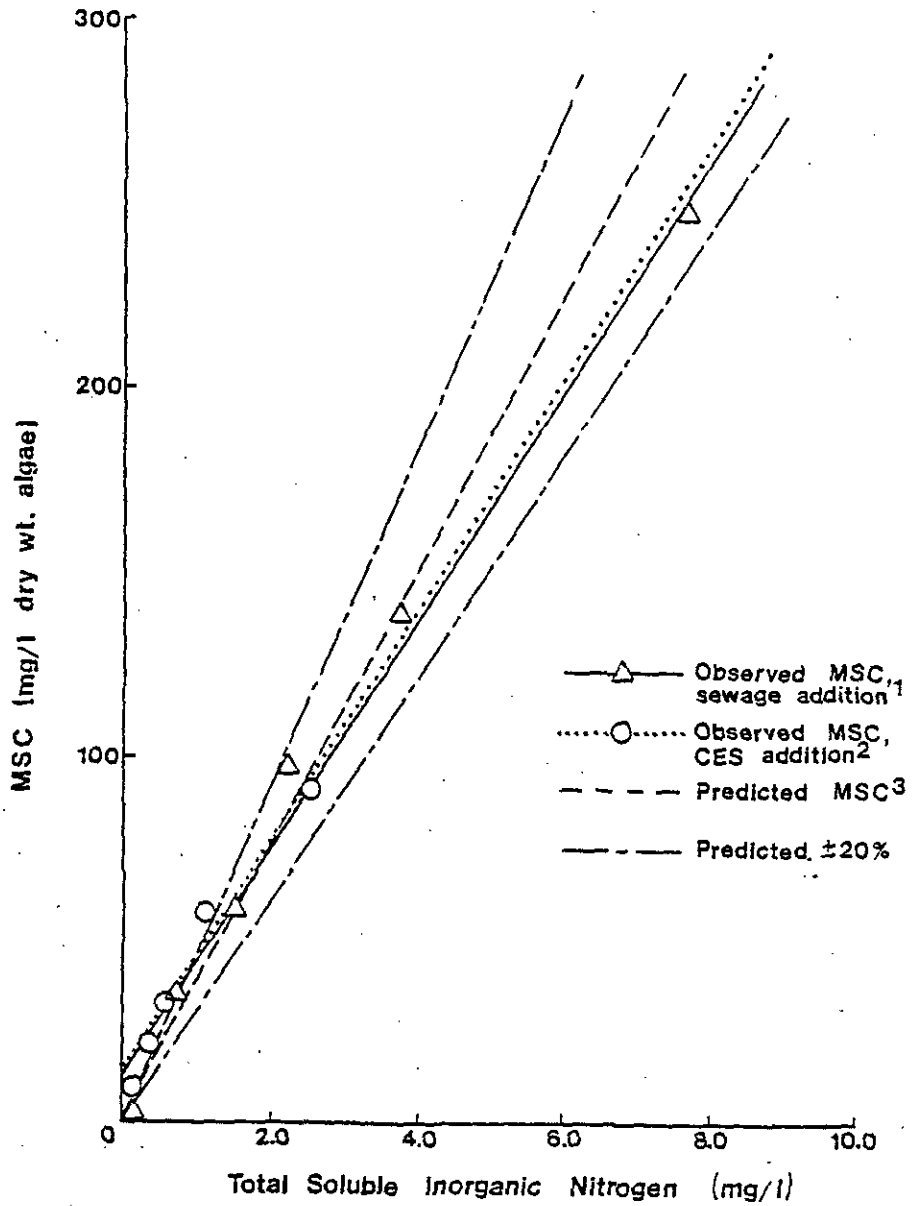
1. CES = chemical equivalent solution
2. Due to error in preliminary analysis of sewage, CES contains approximately half the TSIN (total soluble inorganic nitrogen) contained in the sewage.
3. Predicted yield = 38 X TSIN (mg/l) $\pm$ 20%; $\pm$ 20% range is indicated by values in parentheses.

Figure 3-9. Observed and predicted MSC (mg/l dry wt.) for Selenastrum capricornutum grown in Mill River water sampled July 16, 1981 plus chemical equivalent solution (CES) additions



1. Observed MSC (mg/l dry wt.) = 30.48 X TSIN (mg/l)+ 11.94 (11)
2. Predicted MSC (mg/l dry wt.) = 38.0 X TSIN (mg/l) ± 20% (2)

Figure 3-10. Observed and predicted MSC (mg/l dry wt.) for Selenastrum capricornutum grown in Mill River water sampled July 16, 1981 plus sewage and chemical equivalent solution (CES) additions.



1. Observed MSC (mg/l dry wt.) = 30.54 X TSIN (mg/l) + 11.54 (10)
2. Observed MSC (mg/l dry wt.) = 30.48 X TSIN (mg/l) + 11.94 (11)
3. Predicted MSC (mg/l dry wt.) = 38.0 X TSIN (mg/l) ± 20 % (2)

Table 3-11. Effect of EDTA and micronutrient additions on Selenastrum capricornutum grown in Mill River water sampled July 16, 1981 with chemical equivalent solution (CES) additions.

Mill River water + addition (by volume)	MSC (mg/l dry wt.)	t_c [1]	$t_{.995}$ [2]	H_o: $X_1=X_2$
5% CES	20.70	- 2.75	3.25	Accept[3]
5% CES, EDTA	21.84			
5% CES	20.70	+ 0.34	3.25	Accept
5% CES, micronutrients	20.49			
10% CES	33.29	+0.67	3.25	Accept
10% CES micronutrients	32.63			
10% CES	33.29	-2.48	2.92	Accept
10% CES, EDTA	38.87			
20% CES	58.04	-2.26	3.17	Accept
20% CES, EDTA	67.27			
20% CES	58.04	+1.45	2.92	Accept
20% CES, micronutrients	56.43			
50% CES	91.12	-2.81	2.92	Accept
50% CES, EDTA	105.53			
50% CES	91.12	+1.42	2.92	Accept
50% CES, micronutrients	82.44			

1. $t_c = t_{calculated}$ using equation (6) or (7)
2. $t_{.995}$ varies depending upon calculated degrees of freedom.
3. accept hypothesis $X_1 = X_2$ (MSC values are not statistically different) when $t_{.995}$ is less than $|t_c|$.

Lime treated sewage and lime treated chemical equivalent solution additions to Mill River water sampled July 16, 1981

Secondary treated sewage and chemical equivalent solution of the sewage were both treated with lime to remove inorganic phosphorus. Lime treatment reduced orthophosphorus in the sewage from 2.976 mg/l to 0.003 mg/l. Lime treatment removed virtually 100 % of the 2.968 mg/l orthophosphorus present in the chemical equivalent solution to a level below the limits of detection. Total phosphorus analysis of treated sewage and treated chemical equivalent solution were not performed. The great degree of orthophosphorus removal shifted algal cultures from nitrogen to phosphorus limitation. Predicted and observed algal MSC values for varying additions of both treated sewage and treated chemical equivalent solution of the sewage are presented in Table 3-12. Observed growth is plotted versus percentage treated addition in Figures 3-11 and 3-12. Both the treated sewage and the treated chemical equivalent solution had lower phosphorus content that the Mill River diluent. Predicted MSC values therefore decreased with increasing sewage or equivalent solution addition as the phosphorus level in the diluent Mill River water decreased by dilution.

The observed MSC values for both the 5 % and 10 % additions of the treated chemical equivalent solution and for the sewage were greater than that predicted by equation 3. This may reflect some error in the chemical analyses. The observed MSC values for the 20 % addition of both the sewage and chemical equivalent solution were considerably lower than the value predicted by equation 3 indicating the presence of some toxicant, or the absence of some other limiting compound.

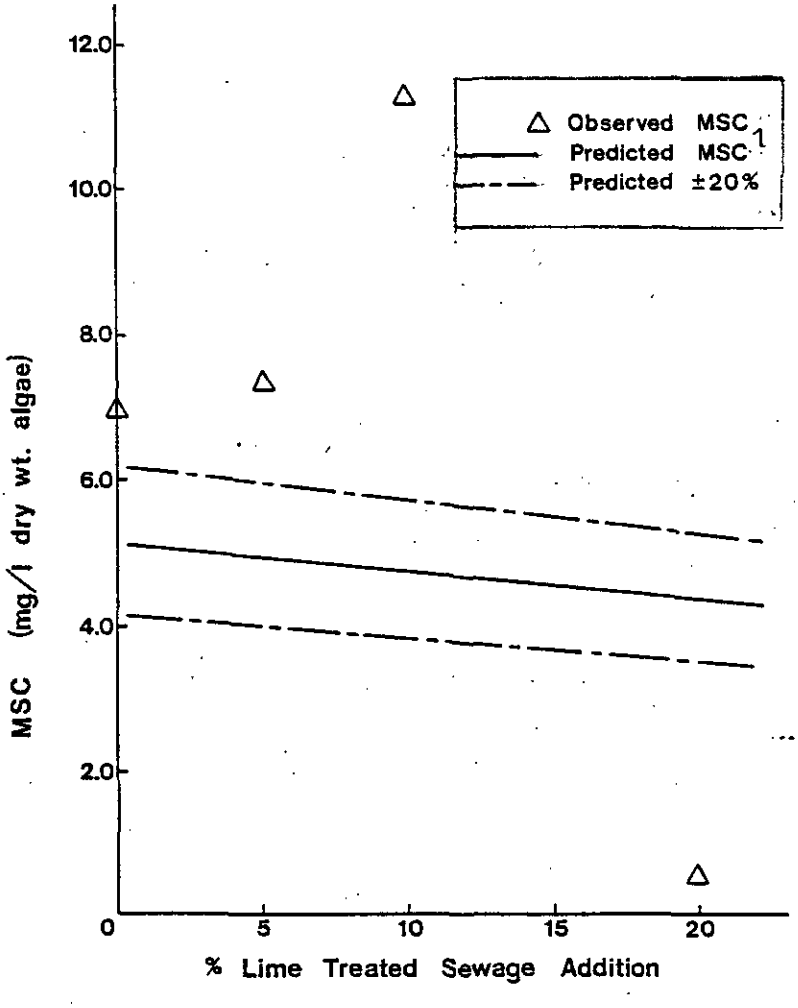
Table 3-12. Predicted and observed MSC (mg/l dry wt.) of Selenastrum capricornutum grown in Mill River water sampled July 16, 1981, plus lime-treated sewage and lime-treated chemical equivalent solution.

Mill River plus + treated addition	Predicted MSC[1] (mg/l dry wt.)	Observed MSC (mg/l dry wt.)
no addition	3.67 (2.94- 4.40)	7.09
+ 5 % sewage	4.96 (3.97- 5.95)	7.26
+ 10 % sewage	4.76 (3.81- 5.71)	11.32
+ 20 % sewage	4.35 (3.48- 5.22)	0.48
+ 5 % CES[2]	4.90 (3.92- 5.88)	11.00
+ 10 % CES	4.64 (3.72- 5.57)	10.40
+ 20 % CES	4.13 (3.30- 4.95)	0.40

1. Predicted yield = 430 X ortho-P (mg/l) $\pm$ 20 % (3) ; $\pm$ 20 % range is indicated by values in parentheses.

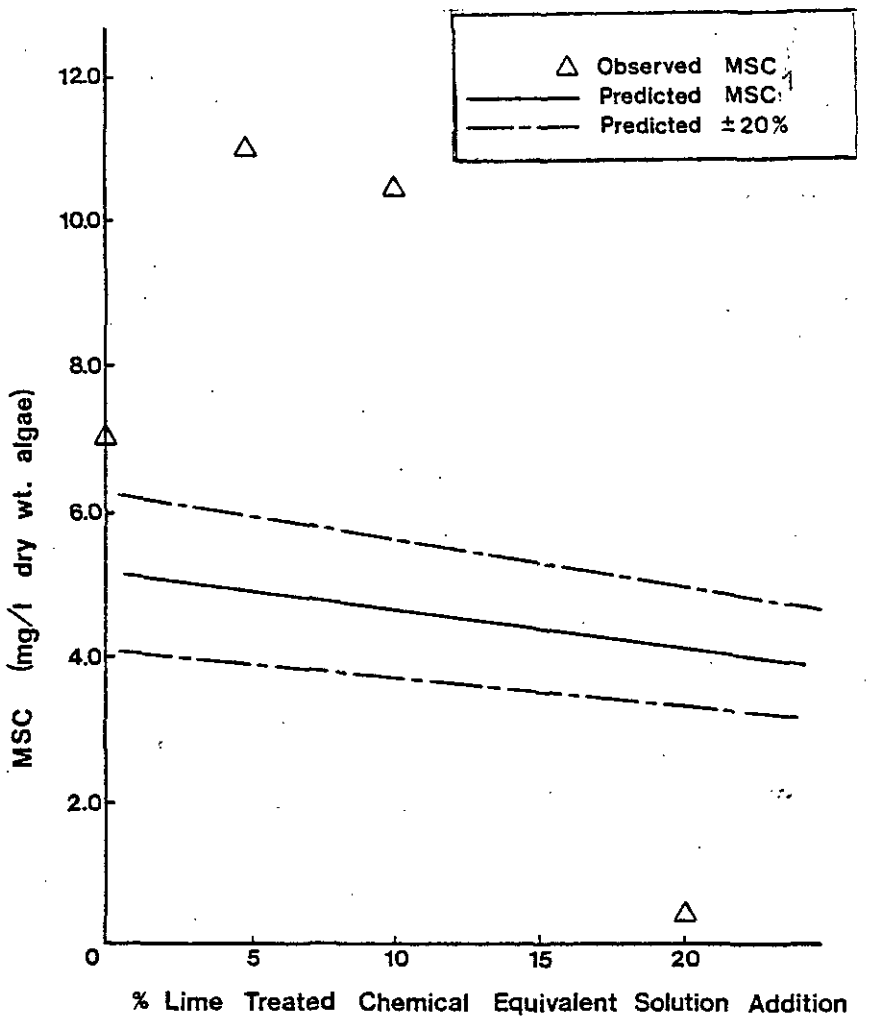
2. CES = chemical equivalent solution.

Figure 3-11. Observed and predicted MSC (mg/l dry wt.) for Selenastrum capricornutum grown in Mill River water sampled July 16, 1981 plus lime-treated sewage additions.



1. Predicted MSC (mg/l dry wt.) = 430 X ortho-P (mg/l) ± 20 % (3)

Figure 3-12. Observed and predicted MSC (mg/l dry wt.) for Selenastrum capricornutum grown in Mill River water sampled July 16, 1981 plus lime-treated chemical equivalent solution additions.



1. Predicted MSC (mg/l dry wt.) = 430 X ortho-P (mg/l) ± 20% (3)

Sewage additions to Mill River water sampled September 27, 1981

An algal assay was performed using additions of sewage collected September 27 to Mill River water, also sampled September 27, 1981. Chemical analyses of the secondary treated sewage effluent are presented in Table 3-5. The resulting algal growth is reported in Table 3-13 and Table A-10 in the Appendix. Additions of 10 %, 15 %, 25 %, and 50 % sewage resulted in maximum standing crop values within the 20% predicted MSC range but the 5 % sewage addition without EDTA was just outside the predicted range.

A plot of algal growth versus TSIN and percent sewage additions is shown in Figure 3-13. Algal growth was nitrogen limited at all sewage addition levels. The linear regression of the observed data was:

MSC (mg/l dry wt.) = 32.05 X TSIN (mg/l) + 10.83 (12)

with an r value of 0.9945.

Statistical analysis was performed on the data to examine the effect of EDTA additions on algal growth. The results are presented in Table 3-14. The difference in MSC values for each level of addition between samples with and without EDTA additions was significant only at the 5 % addition level. The 5 % sewage plus EDTA sample resulted in a lower MSC value than the corresponding sample without EDTA. EDTA did not therefore significantly aid algal growth even at the 5 % dilution. There is no evidence of either toxicity or micronutrient limitation.

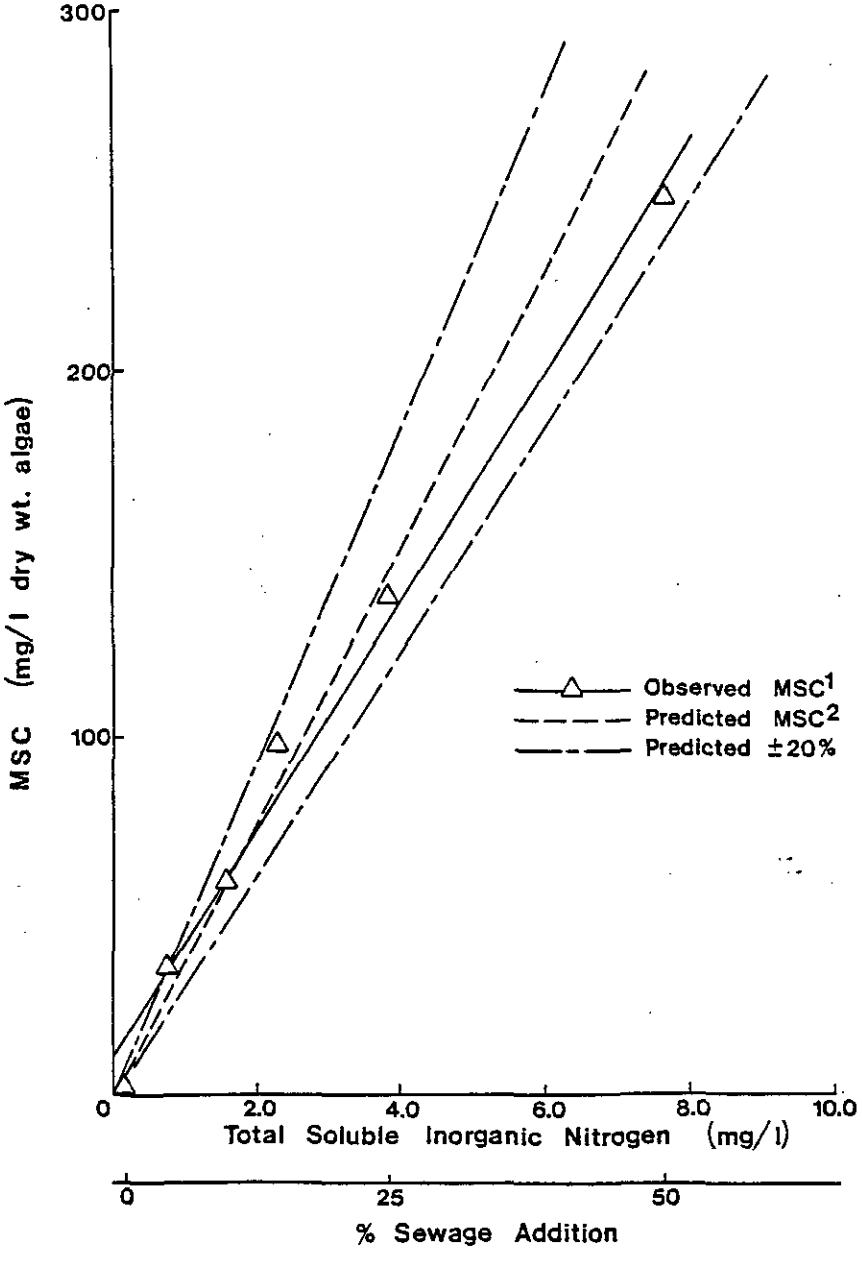
Table 3-13. Predicted and observed MSC (mg/l dry wt.) of Selenastrum capricornutum grown in Mill River water plus sewage additions, both sampled September 27, 1981.

Mill River water plus	Predicted MSC[1] (mg/l dry wt.)	Observed MSC (mg/l dry wt.)
no addition	0.59 (0.47- 0.71)[2]	0.32
+ 5 % sewage	29.31 (23.45- 35.17)	35.71
+ 5 % sewage, EDTA		33.67
+ 10 % sewage	58.04 (46.43- 69.65)	57.66
+ 10 % sewage, EDTA		62.41
+ 15 % sewage	86.76 (69.41-104.10)	97.87
+ 15 % sewage, EDTA		95.94
+ 25 % sewage	144.21 (115.37-173.06)	139.20
+ 25 % sewage, EDTA		142.40
+ 50 % sewage	287.84 (230.27-345.41)	246.53
+ 50 % sewage, EDTA		248.69

1. Predicted yield = 38 X TSIN ± 20 % (2)

2. Values in parentheses represent acceptable range (± 20 %) of predicted values.

Figure 3-13. Observed and predicted MSC (mg/l dry wt.) Selenastrum capricornutum grown in Mill River water plus sewage additions, both sampled September 27, 1981.



1. Observed MSC (mg/l dry wt.) = 32.05 X TSIN (mg/l) + 10.83 (12)
2. Predicted MSC (mg/l dry wt.) = 38.0 X TSIN (mg/l) ± 20 % (2)

Table 3-14. Effect of EDTA additions on Selenastrum capricornutum grown in Mill River water with sewage additions, both sampled September 27, 1981.

Mill River water plus	MSC (mg/l dry wt)	t_c [1]	$t_{.995}$	$H_o: x_1 = x_2$
5 % sewage	35.71	+ 4.51	2.92	Reject [2]
5 % sewage,EDTA	33.67			
10% sewage	57.66	- 1.71	2.92	Accept [3]
10% sewage,EDTA	62.41			
15% sewage	97.84	+ 1.03	2.92	Accept
15% sewage,EDTA	95.94			
25% sewage	139.20	-0.30	2.92	Accept
25% sewage,EDTA	142.41			
50% sewage	246.53	- 0.22	2.92	Accept
50% sewage,EDTA	248.69			

1. $t_c = t_{calculated}$ using equation (6) or (7).
2. Reject hypothesis $x_1 = x_2$ (MSC values are statistically different) when $t_{.995}$ is less than $|t_c|$.
3. Accept hypothesis $x_1 = x_2$ (MSC values are not statistically different) when $t_{.995}$ is greater than $|t_c|$.

Chemical equivalent solution additions to Mill River water sampled September 27, 1981.

Tables 3-15 and A-12 show the observed and predicted yields for additions of chemical solution, equivalent to the inorganic nitrogen and phosphorus content of the sewage sampled on September 27, 1981, to Mill River water aliquots. The MSC values for actual sewage additions are also shown in Table 3-13. The observed MSC values for the chemical equivalent solution were slightly greater than the values predicted by equation (2) at the 5 % and 10 % addition levels. MSC values at 15 % and 25 % additions were within the range of predicted values. Figure 3-14 shows observed MSC values and the predicted algal growth at varying percent additions of the chemical equivalent solution. Algal growth was nitrogen limited at all levels of addition. Linear regression of the observed data gave the following equation for algal yield:

$$\text{MSC (mg/l dry wt.)} = 37.42 \times \text{TSIN (mg/l)} + 8.67 \qquad (13)$$

with an r value of 0.9971.

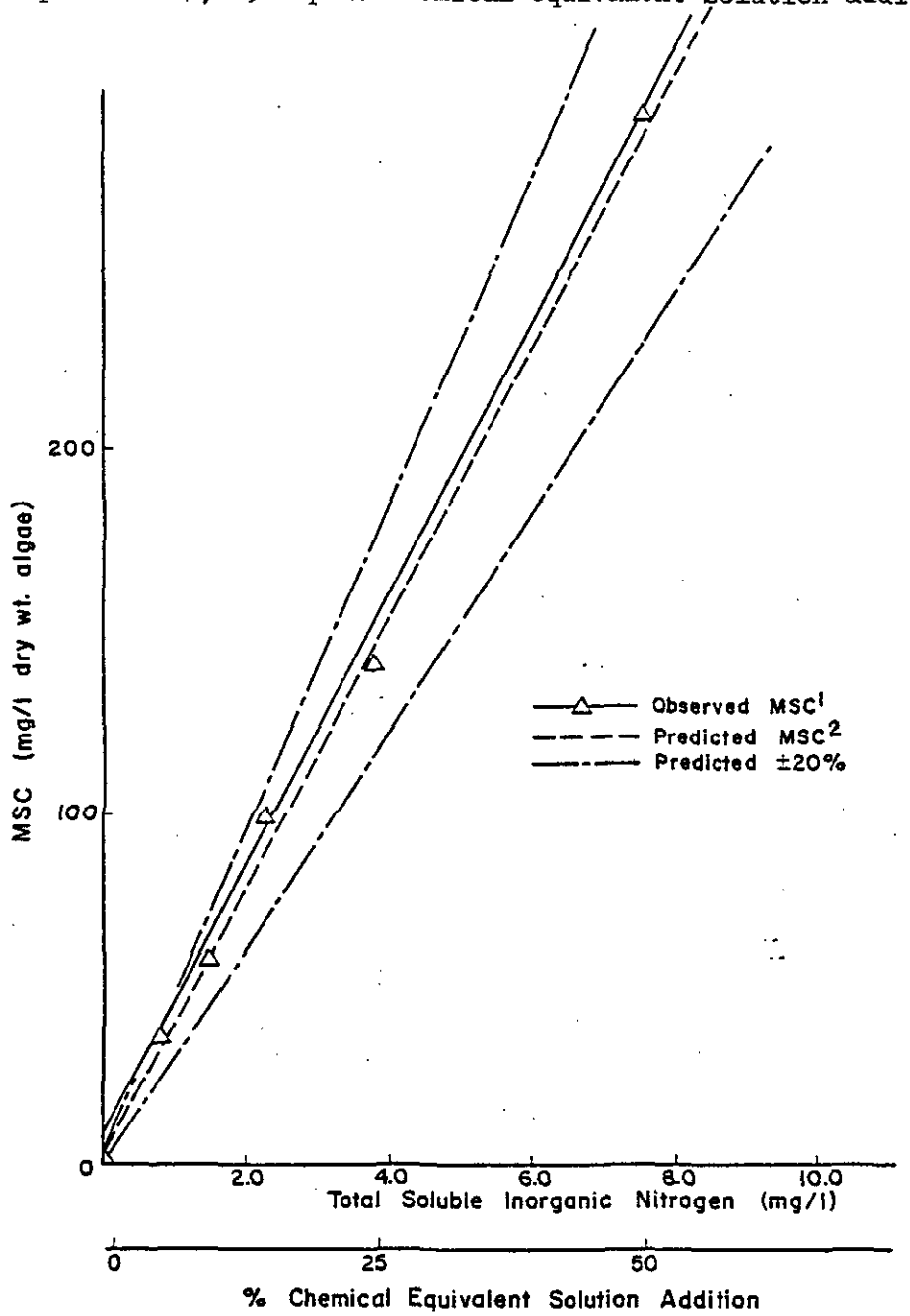
Statistical analysis of the chemical equivalent solution (CES) additions versus CES plus EDTA additions are shown in Table 3-16. Algae grown in solutions containing EDTA spikes attained significantly greater MSC yields, at the 99 % confidence level, than cultures without EDTA additions only at chemical equivalent solution additions of 25 % and 15%.

Table 3-15. Predicted and observed MSC (mg/l dry wt.) of Selenastrum capricornutum grown in Mill River water sampled Sept. 27, 1981 plus chemical equivalent solution (CES) additions.

Mill River water plus	Predicted MSC[1] (mg/l dry wt.)	Observed MSC (mg/l dry wt.)
no addition	0.59 (0.47- 0.71)[2]	0.11
+ 5 % CSE	29.31 (23.45- 35.17)	37.21
+ 5 % CSE, EDTA		38.73
+ 10 % CSE	58.04 (46.43- 69.65)	74.34
+ 10 % CSE, EDTA		73.06
+ 15 % CSE	86.76 (69.41- 104.10)	103.51
+ 15 % CSE, EDTA		113.42
+ 25 % CSE	144.21 (115.37-173.06)	142.80
+ 25 % CSE, EDTA		163.84
+ 50 % CSE	287.84 (230.27-345.41)	291.53
+ 50 % CSE, EDTA		293.34

1. Predicted yield = 38 X TSIN (mg/l) $\pm$ 20 % (2)
2. Values in parentheses represent acceptable range ($\pm$ 20%) of predicted values.

Figure 3-14. Observed and predicted MSC (mg/l dry wt.) Selenastrum capricornutum grown in Mill River water sampled September 27, 1981 plus chemical equivalent solution additions.



1. Observed MSC (mg/l dry wt.) = 37.42 X TSIN (mg/l) + 8.67 (13)
2. Predicted MSC (mg/l dry wt.) = 38.0 X TSIN (mg/l) ± 20 % (2)

Table 3-16. Effect of EDTA additions on Selenastrum capricornutum grown in Mill River water sampled September 27, 1981 with chemical equivalent solution (CES) additions.

Mill River water plus	MSC (mg/l dry wt.)	t_c [1]	$t_{.995}$ [2]	$H_o: x_1 = x_2$
5 % CES	37.21	- 2.84	2.92	Accept [3]
5 % CES, EDTA	38.73			
10 % CES	74.34	+ 1.14	2.92	Accept
10 % CES, EDTA	73.06			
15 % CES	103.51	3.44	3.11	Reject [4]
15 % CES, EDTA	113.42			
25 % CES	142.80	- 4.56	3.17	Reject
25 % CES, EDTA	163.84			
50 % CES	291.53	- 0.30	3.01	Accept
50 % CES, EDTA	293.34			

1. $t_c = t_{calculated}$ using equation (6) or (7).
2. $t_{.995}$ varies depending upon calculated degrees of freedom.
3. Accept hypothesis $x_1 = x_2$ (MSC values are not statistically different) when $t_{.995}$ is greater than $|t_c|$.
4. Reject hypothesis $x_1 = x_2$ (MSC values are statistically different) when $t_{.995}$ is less than $|t_c|$.

Table 3-17, Appendix Tables A-10 and A-12, and Figure 3-15 present data on observed MSC values for algae grown with sewage or chemical equivalent solution additions. There is close agreement of these values at all percentage additions. This is demonstrated by the linear regression analysis of the data. For sewage additions

MSC (mg/l dry wt.) = 32.05 X TSIN (mg/l) + 10.83 (12)

with an r value of 0.9945, and for sewage plus EDTA additions

MSC (mg/l dry wt.) = 32.42 X TSIN(mg/l) + 10.83 (14)

with an r value of 0.9949. For the chemical equivalent solution additions

MSC (mg/l dry wt.) = 37.42 X TSIN (mg/l) + 8.67 (13)

with an r value of 0.9971, and for chemical equivalent solutions plus EDTA additions

MSC (mg/l dry wt.) = 38.14 X TSIN (mg/l) + 12.10 (15)

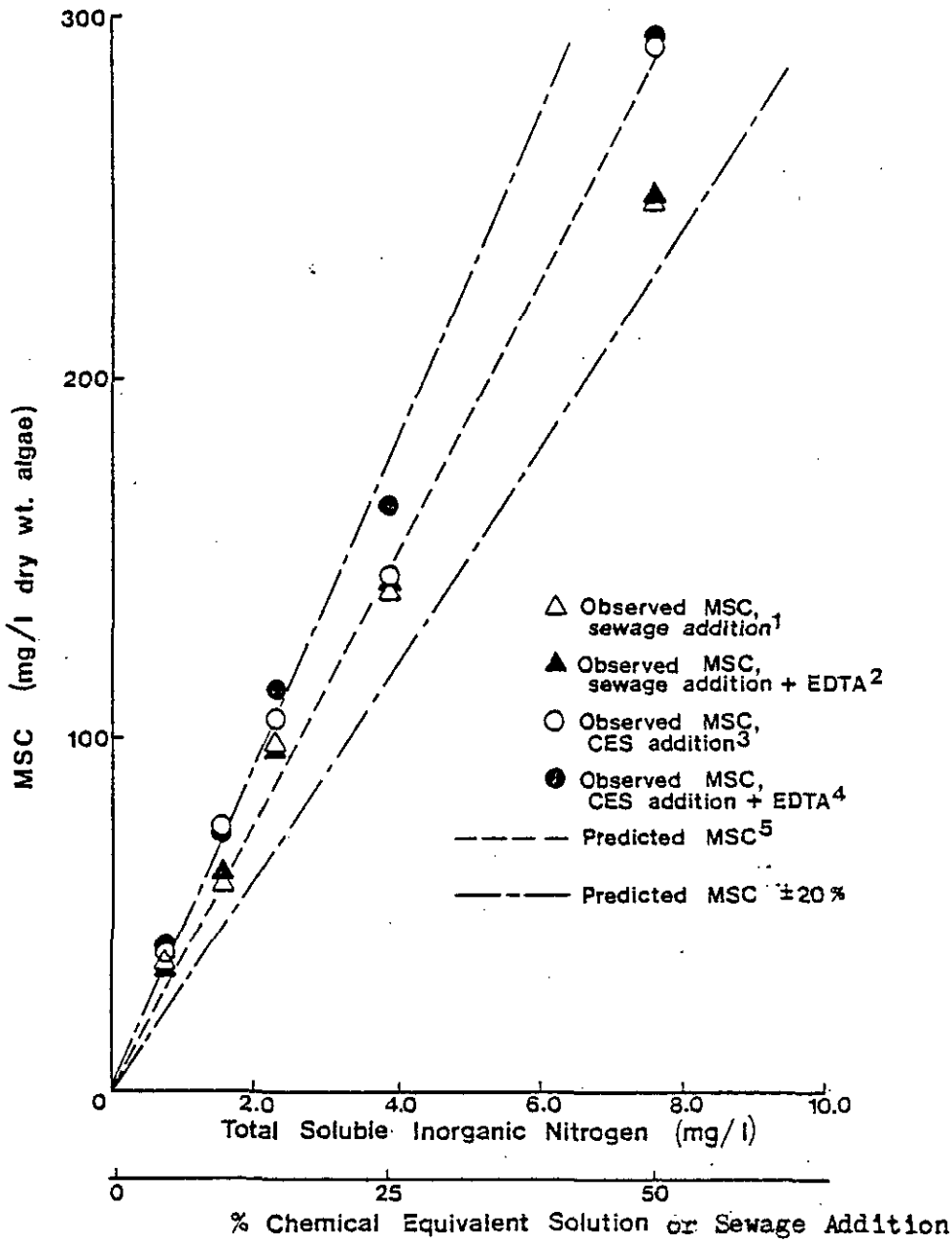
with an r value of 0.9954.

A statistical comparison between sewage MSC values and chemical equivalent solution MSC values is shown in Table 3-18. A statistical difference between these two treatments was observed only at the 10 % and 50 % addition levels. In both cases higher yields were observed for chemical equivalent solution additions than for sewage additions. These data demonstrate that algal response to sewage addition may be attributed to the inorganic nitrogen and phosphorus content of this addition.

Table 3-17. Observed MSC (mg/l dry wt.) of Selenastrum capricornutum grown in Mill River water sampled September 27, 1981 with sewage or chemical equivalent solution (CES) additions.

% Addition	MSC (mg/l dry weight)			
	Sewage	Sewage+EDTA	CES	CES+EDTA
0	0.32		0.11	
5	35.71	33.67	37.21	38.73
10	57.66	62.41	74.34	73.06
15	97.87	95.94	103.51	113.42
25	139.20	142.40	142.80	163.84
50	246.53	248.69	291.53	293.34

Figure 3-15. Observed and predicted MSC (mg/l dry wt.) Selenastrum capricornutum grown in Mill River water sampled Sept. 27, 1981 plus sewage and chemical equivalent solution additions.



1. Observed MSC (mg/l dry wt.) = 32.05 X TSIN (mg/l) + 10.83 (12)
2. Observed MSC (mg/l dry wt.) = 32.42 X TSIN (mg/l) + 10.84 (14)
3. Observed MSC (mg/l dry wt.) = 37.42 X TSIN (mg/l) + 8.67 (13)
4. Observed MSC (mg/l dry wt.) = 38.14 X TSIN (mg/l) + 12.10 (15)
5. Predicted MSC (mg/l dry wt.) = 38.0 X TSIN (mg/l) ± 20 % (2)

Table 3-18. Comparison of the algal growth response of Selenastrum capricornutum grown in Mill River water sampled Sept. 27, 1981 to varying percentage additions of sewage versus chemical equivalent solution (CES).

Mill River water plus	MSC (mg/l dry wt.)	t_c [1]	$t_{.995}$ [2]	$H_o: x_1 = x_2$
5 % sewage 5 % CES	35.71 37.21	- 2.91	2.92	Accept [3]
10 % sewage 10 % CES	57.66 74.34	- 6.53	2.92	Reject [4]
15 % sewage 15 % CES	97.87 103.51	- 1.95	2.92	Accept
25 % sewage 25 % CES	139.20 142.80	- 0.45	2.92	Accept
50 % sewage 50 % CES	246.53 291.53	- 4.56	3.01	Reject

1. $t_c = t_{calculated}$ using equation (6) or (7).
2. $t_{.995}$ varies depending upon calculated degrees of freedom.
3. Accept hypothesis $x_1 = x_2$ (MSC values are not statistically different) when $t_{.995}$ is greater than $|t_c|$.
4. Reject hypothesis $x_1 = x_2$ (MSC values are statistically different) when $t_{.995}$ is less than $|t_c|$.

Alum-treated sewage and alum-treated chemical equivalent solution additions to Mill River water

Sewage samples collected on September 27 and a chemical equivalent solution of this sewage were each treated with alum to remove phosphorus. The alum dosage was calculated incorrectly, and the sewage and chemical equivalent solution were treated with an Al:P molar dosage of approximately 3:1 rather than the 1.5:1 molar ratio originally intended. Predicted removal for this level of treatment is greater than 95% (EPA, 1976). In both cases over 99 % removal was achieved. The orthophosphorus level in treated sewage was 9.1 ug/l, and 15.2 ug/l in treated equivalent solution. A similar level of phosphorus reduction was achieved in the sewage, where total phosphorus was reduced from 4.72 mg/l to 36.0 ug/l. This was greater than 99 % reduction of the total phosphorus. As the chemical equivalent solution contained only orthophosphorus, reduction in total phosphorus was the same as orthophosphorus reduction.

Table 3-19 shows the predicted and observed MSC values for algae grown in treated sewage or chemical equivalent solution additions to Mill River water. These cultures contained so few algal colonies that determination of MSC values using the Coulter Counter resulted in values which were very close to the instrument's limit of detection, and thus not as reliable as higher readings. However, in all cases, observed yields were much lower than predicted values. Later algal assay on alum treated sewage (Plotkin and Ram, 1981) demonstrated that alum was not toxic at high addition levels. It is therefore unlikely that alum toxicity caused low algal MSC values.

Table 3-19. Observed and predicted algal MSC values (mg/l dry wt.) for Selenastrum capricornutum grown in Mill River water with varying additions of either alum-treated sewage or alum-treated chemical equivalent (CES) solution.

Addition	Predicted MSC[1] (mg/l dry wt.)	Observed MSC (mg/l dry wt.)
5 % sewage	1.41 (1.13-1.69)	0.13
10 % sewage	1.54 (1.23-1.85)	0.11
15 % sewage	1.67 (1.34-2.01)	0.17
25 % sewage	1.95 (1.56-2.35)	0.15
50 % sewage	2.63 (2.10-3.16)	0.19
5 % CES	1.54 (1.23-1.85)	0.10
10 % CES	1.80 (1.44-2.16)	0.14
15 % CES	2.07 (1.65-2.48)	0.25
25 % CES	2.60 (2.07-3.11)	0.17
50 % CES	3.91 (3.13-4.70)	0.70

1. Predicted yield (mg/l dry wt.) = 430 X ortho-P (mg/l) $\pm$ 20 % (3). Values in parentheses represent the acceptable range of predicted values ($\pm$ 20 %).

CHAPTER 4

DISCUSSION

Nutrient Status of the Mill River

Results from chemical analyses and nutrient limitation algal assays of water sampled from the Mill River illustrate the value of using algal assay to assess the nutrient status of natural waters. Chemical analyses of phosphorus levels in the Mill River samples revealed N:P ratios below 9 in all cases (3.75, 8.33, 5.33) initially indicating the waters were nitrogen limited for algal growth. Nutrient limitation studies using the algal assay bottle test confirmed that algal growth was nitrogen limited in the water sample collected on June 24, 1981. However, algal assays for water sampled July 16, 1981, and September 27, 1981 exhibited co-limitation by nitrogen and phosphorus, and phosphorus limitation, respectively.

There is no single value for the N:P ratio which indicates a shift of algal growth limitation from nitrogen to phosphorus. Miller et al. (1978) defined a water to be nitrogen limited for algal growth when the N:P ratio was less than 11:1, and phosphorus limited for growth when the N:P ratio was greater than 11:1. Weiss (1976), however reported waters to be nitrogen limited for algal growth when the N:P ratio was less than 8:1, phosphorus limited for growth when N:P ratios were greater than 13:1, and phosphorus and nitrogen co-limited when N:P ratios were between 9:1 and 12:1. Similarly, Chiaudani and Vighi (1976) found natural waters to be growth limited by both nitrogen and phosphorus when N:P ratios were

between 5:1 and 10:1, nitrogen limited for growth below a ratio of 5:1 and phosphorus limited for growth above a N:P value of 10:1. Thus it is possible the chemical analyses of Mill River water sampled July 24 was correct, and that the water was co-limited for algal growth by nitrogen and phosphorus with a N:P ratio of 8.33:1.0 .

The Mill River water sampled September 27 had nutrient levels just above the level of detection. The algal assay was a valuable method by which these analyses were checked. The nutrient limitation study of this river sample showed algal growth to be phosphorus limited, although the N:P ratio was determined to be 5.33:1.0 . An error in chemical analyses of the Mill River due to the extremely low nutrient levels likely ocurred.

The Mill River proved to be a good source of dilution water for this study. Although the nutrient content and the limiting nutrient of the sample water varied, added sewage or chemical equivalent solution contained high enough nitrogen and phosphorus levels for algal growth to be always nitrogen limited. This facilitated comparisons of assays using different diluent samples. Similarly, additions of secondary treated sewage or its chemical equivalent solution, after treatment with alum or lime to remove phosphorus, caused the resulting algal cultures to be phosphorus limited for growth at all levels of addition. Furthermore, there were no instances where algal MSC values were significantly lower than predicted algal yield which could have been attributed to toxicants or micronutrient limitation in Mill River water.

Stimulatory Effect of Secondary Treated Sewage

Addition of secondary treated wastewater effluent to Mill River water samples caused nitrogen limited algal growth in all cases. The algal assay of sewage additions to Mill River water sampled June 24 showed consistantly higher MSC values than could be predicted based on total soluble inorganic nitrogen (TSIN) levels alone, or TSIN plus total organic nitrogen (TON) levels. There are several possible hypotheses for this phenomenon. There may have been some experimental error in the analytical determination of TSIN or in performing the algal assay. Alternatively, the acceptable limits to which Selenastrum capricornutum may be able to utilize TSIN in the production of cell biomass may be greater than the ± 20% range indicated by equation 2 . It is also possible that organic nitrogen not determined by the total Kjeldahl-N technique such as azide, azo, hydrozone, nitrile, nitro, nitroso, oxime, or semicarbazone nitrogen forms (Standard Methods, 1980) contributed to the higher levels of algal growth.

The results of two additional algal assays in which secondary treated wastewater was added to Mill River water sampled July 16 and September 27 differed from results of the first assay. The observed MSC values were all within the ± 20 % range of the predicted values and thus algal growth could be completely accounted for the by the TSIN. It seems unlikely then, that the secondary treated wastewater contained any significant levels of compounds, other than inorganic nitrogen and orthophosphorus, which contributed to algal growth.

That algal growth can be completely accounted for by inorganic nitrogen and orthophosphorus was further corroborated by algal assays with sewage chemical equivalent solution additions. Additions of a chemical solution equivalent to the inorganic nitrogen and phosphorus content of the Amherst wastewater to Mill River water sampled on July 16 resulted in MSC values which were slightly above the predicted yields at three of the four percentage levels of addition. Figure 3-10 shows the close agreement of observed algal yields for chemical equivalent solution (CES) and sewage additions. The equations of the lines through these data points were virtually identical:

$$\text{MSC (mg/l dry wt.)}_{\text{sewage addition}} = 30.54 \times \text{TSIN (mg/l)} + 11.54 \qquad (10)$$

$$\text{MSC (mg/l dry wt.)}_{\text{CES addition}} = 30.48 \times \text{TSIN (mg/l)} + 11.94 \qquad (11)$$

Thus it seems unlikely that there were any additional algal growth nutrients in the sewage which were not contained in the chemical equivalent solution.

Varying percent additions of chemical equivalent solutions to Mill River water sampled September 27 exhibited similar results. Observed MSC values were slightly higher than predicted values at two of the five addition levels. Figure 3-15 shows the correlation of observed MSC values for sewage and chemical equivalent of sewage. Again the equations of the two lines passing through the data points for these two additions were in close agreement with each other:

$$\text{MSC (mg/l dry wt.) sewage addition} = 32.05 \times \text{TSIN (mg/l)} + 10.83 \quad (12)$$

$$\text{MSC (mg/l dry wt.) CES addition} = 37.42 \times \text{TSIN (mg/l)} + 8.67 \quad (13)$$

The low organic nitrogen and moderate organic phosphorus levels in the effluent used in these algal assays must be considered in evaluating the significance of data obtained from the addition of sewage or chemical equivalent solution to Mill River water. The low organic nutrient level in the sewage is partially attributable to the required filtration of the sewage prior to algal assay which removes particulate material containing organic phosphorus and nitrogen. The organic phosphorus level in the sewage comprised 25 %, 6 %, and 14 % of total phosphorus levels in the three samplings of Amherst wastewater treatment effluent. Organic nitrogen comprised only 5 %, 4 %, and 5 % of the total nitrogen content of the sewage in the three samplings. Actual TON levels may have actually been greater than the values determined by Kjeldahl-N analysis owing to the inability of this chemical analysis to determine certain nitrogen forms.

Algal growth observed in varying percentage additions of sewage to Mill River water were generally equal to or slightly less than that predicted by the inorganic nitrogen level of the sewage (Fig. 3-8,3-13) suggesting that the smaller percentage of organic nitrogen did not contribute significantly to observed algal growth.

This finding is not conclusive, however, owing to the very small percentage of organic nitrogen and the nitrogen limited status of the sewage. The very close agreement between observed and predicted algal yields also indicated the absence of both toxicants and micronutrient limitation in the effluent. The TSIN alone, therefore, appeared to be an excellent method for predicting the stimulatory response of the test algal species to the sewage addition. Inclusion of the TON in the predicted yield equation, i.e. MSC = 38 X (TON + TSIN), would further support this hypothesis since the observed algal growth would fall somewhat further below the predicted yields. The finding that organic nitrogen did not contribute to the observed algal yield was further demonstrated in the additions of chemical equivalent solution to algal cultures. Observed algal yields for these studies were identical to the algal growth response in algal cultures receiving actual sewage in one case, and were somewhat greater than the actual sewage in a second case. Thus the chemical solution containing inorganic nutrients alone resulted in a growth response equal to or greater than that of the sewage additions. Such a response would not have been expected if the organic nitrogen 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 other studies.

The contribution of organic phosphorus to algal growth in these samples could not be assessed since the sewage was always nitrogen limited.

While these studies indicate that low levels of organic nitrogen in a secondary treated municipal wastewater did not stimulate algal growth, additional studies using effluents containing larger organic nutrient levels are needed to further clarify the role of these compounds on algal growth stimulation in environmental waters.

EDTA and micronutrient additions promoted greater growth of Selenastrum capricornutum to an extent which was statistically significant, at the 99 % confidence level, in only 4 out of 20 sets of sewage addition cultures (Tables 3-7, 3-9, and 3-14) and in only 2 out of 13 sets of chemical equivalent solution addition cultures (Tables 3-11 and 3-16). It is therefore unlikely that the sewage sampled from the Amherst wastewater treatment plant contained toxicants or was lacking in micronutrients. However, it would be unwise to generalize such results and presume that EDTA and micronutrient additions are unnecessary in other algal assays of sewage effluent due to the variability of wastewater content.

Treatment to Remove Phosphorus

Both alum and lime were used to remove phosphorus from secondary treated wastewater. In both cases the removal of phosphorus was great enough to shift algal cultures to phosphorus limited growth.

Lime treated sewage and lime treated chemical equivalent solution both displayed much lower algal growth than in cultures containing untreated additions. However, algal yields did not agree with predicted MSC values in both the 5 % and 10 % addition levels

of both lime treated sewage and lime treated chemical equivalent solution. These observed algal yields were greater than the predicted values. This might be attributed to error in phosphorus analysis. Both lime treated additions at the 20 % level resulted in algal MSC values greatly below the predicted values, suggesting toxicity or severe nutrient limitation at this level of addition. There did not seem to be any significant difference in the effect of lime treated sewage additions and lime treated chemical equivalent solution additions on algal growth. Further algal assay research is needed using lime treated additions to better understand the biostimulatory properties of treated sewage.

Municipal wastewater and a chemical equivalent solution were also treated with alum to remove phosphorus. Again, a high degree of phosphorus removal caused algal growth to be phosphorus limited. Alum treatment of the sewage and the chemical equivalent solution resulted in virtually 100 % reduction of algal growth. It should be noted that the Al:P molar ratio used for this study was not typical of the degree of phosphorus removal usually achieved by conventional tertiary wastewater treatment. Residual phosphorus levels are normally in the range of 1 mg/l. A certain degree of algal growth attributable to additions of such treated wastewater is normally likely.

The observed yields for both treated sewage and treated chemical equivalent solution were much lower than the predicted values. Preliminary studies by Plotkin and Ram (1981) have observed

that alum is nontoxic to algal growth. It is likely, therefore, that the very low MSC values were attributable to severe nutrient limitation. Further studies using additions of alum treated sewage with phosphorus levels typical of tertiary treated effluents would be useful in further assessing the ability of alum treatment to reduce algal proliferation in receiving waters.

The research conducted here has shown the algal assay bottle test to be a useful research tool in evaluating the bioavailable nutrients in natural waters and wastewater effluents. This technique was successfully used to assess the nutrient limitation status of three water samples collected from the Mill River. The studies demonstrated that algal growth resulting from either secondary treated wastewater effluent or a chemical equivalent solution of the effluent could be predicted from the orthophosphorus and inorganic nitrogen levels of the additions. While this conclusion is valid for the wastewater examined in this study, additional research is needed on other wastewaters to determine whether this finding is true for municipal wastewaters in general. Treatment with lime and alum caused significant reduction in biostimulatory properties of the sewage and its chemical equivalent.

CHAPTER 5

SUMMARY

Algal assays were conducted using Selenastrum capricornutum, following the procedures outlined by Miller et al. (1978).

1. Water sampled from the Mill River, Amherst, Massachusetts, was used as dilution water in this study. Nutrient limitation studies of this water sampled June 24, 1981, July 16, 1981, and September 27, 1981 showed algal growth to be nitrogen limited, phosphorus and nitrogen co-limited, and phosphorus limited, respectively. Growth limitation by both nitrogen and phosphorus, as determined by algal assay technique, was observed at an N:P ratio of as low as 8.33:1.0 .
2. A preliminary algal assay using secondary treated sewage additions from the Amherst wastewater treatment plant, Amherst, Massachusetts, resulted in observed algal maximum standing crop values greater than could be accounted for by the chemically determined inorganic plus organic nitrogen and orthophosphorus levels. Observed maximum standing crop values greater than that predicted by both TSIN and TON content may be attributed to : a) experimental error in analytical determinations, b) organic nitrogen compounds, not measurable by Kjeldahl digestion, contributing to the algal yield or, c) utilization by Selenastrum capricornutum of TSIN for the production of cell biomass to an extent greater than that given by equation 2 .

3. Subsequent algal assays using sewage additions resulted in observed algal yields which were in close agreement with predicted yields.
4. Chemical equivalent solutions containing inorganic nitrogen and orthophosphorus in amounts equal to that in the sewage were used in algal assays. The resulting growth did not differ significantly from algal growth caused by the equivalent sewage additions.
5. Algal growth, for the secondary treated wastewater studied here, can be predicted by inorganic nitrogen and orthophosphorus levels alone. Organic constituents did not significantly contribute to the algal growth of Selenastrum capricornutum for the wastewater tested.
6. Treatment of sewage and chemical equivalent solution with lime or alum to remove phosphorus caused significant reduction in observed algal maximum standing crop values. Based upon this study, it appears that advanced wastewater treatment practices should be primarily directed towards removing inorganic nutrients from wastewater prior to discharging into environmental waters.
7. Further studies are needed using additions of alum treated sewage or an alum treated chemically equivalent solution, with residual phosphorus levels typical of tertiary treated wastewater to more completely identify the wastewater components contributing to eutrophication of receiving waters.

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APPENDIX

Table A-1. MSC (mg/l dry wt.) Mill River water sampled 6/24/81 plus sewage additions and nutrient spike additions.

ADDITIONS	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	
CONTROL	6.32	6.41	6.29	6.49	6.36	6.62				μ 6.42
								$s=0$	123.	s 0.015
										$s/\sqrt{m}=.005$
+P	7.04	6.83	6.97	6.82	6.62	6.76	6.63	6.88	6.73	μ: 6.80
										s: .14199
										$s/\sqrt{m}$: .047
+N	29.47	29.81	29.61	29.55	29.36	29.17	28.74	27.76	28.22	$\bar{x}$: 29.08
										s: .6956
										$s/\sqrt{m}$: .722
+N+P	41.45	42.00	41.73	43.39	41.97	43.66	44.21	42.78	42.76	$\bar{x}$ = 42.36
										s: .949
										$s/\sqrt{m}$: .313
+EDTA	7.51	7.71	7.61	8.19	7.98	8.08	7.67	7.95	7.67	$\bar{x}$: 7.82
										s: .237
+ P+EDTA	8.1	7.81	8.15	7.51	7.62	7.62	7.51	7.25	7.57	$\bar{x}$: 7.69
										s: .291
+N+EDTA	40.93	40.93	40.25	39.65	40.93	38.38	39.71	39.09	39.71	$\bar{x}$: 40.02
										s: .776
+P +N +EDTA	42.24	42.78	42.51	41.97	40.84	41.70	44.21	illegible	illegible	$\bar{x}$ illegible
										illegible
+MICRO	7.15	7.52	6.99	7.04	6.94	7.04	7.11	7.16	7.11	$\bar{x}$: 7.12
										s: .166
										$s/\sqrt{m}$: .056
1% SEW	10.32	10.13	10.16	10.28	10.28	10.32	10.43	10.26	10.22	$\bar{x}$: 10.24
										s: .126
										$s/\sqrt{m}$: .042
1% +EDTA	11.89	12.21	11.78	10.51	10.62	10.57	10.78	11.0	10.62	$\bar{x}$: 11.11
										s: .661
										$s/\sqrt{m}$: .220
5% SEW	26.54	26.93	26.54	29.82	29.82	29.17	25.05	24.81	24.81	$\bar{x}$: 27.0
										$s/\sqrt{m}$: .093 s: 2.02
5% +EDTA	26.47	26.59	26.36	25.89	26.23	25.89	24.97	26.0	24.97	$\bar{x}$: 25.93
										s: .599
										$s/\sqrt{m}$: .20
10% SEW	45.38	45.09	45.96	45.67	45.96	46.81	42.88	43.54	42.53	$\bar{x}$: 44.30
										s: 1.48
										$s/\sqrt{m}$: .493
10% +EDTA	45.62	45.92	45.62	42.54	41.92	42.54	43.95	43.85	43.85	$\bar{x}$: 43.97
										s: 1.48
										$s/\sqrt{m}$: .493
30% SEW	126.43	122.43	120.39	121.56	119.71	119.03	123.79	126.43	122.43	$\bar{x}$: 122.47
										s: 2.69
										$s/\sqrt{m}$ .897
30% +EDTA	125.04	123.11	124.47	121.83	121.83	122.49	119.83	121.16	121.16	$\bar{x}$: 122.3
										s: 1.66
										$s/\sqrt{m}$: .553

Table A-1. continued.

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	Y_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	$\bar{X}$
50% SEW	191.36	177.14	179.35	182.87	177.14	179.61	191.02	183.32	184.08	$\bar{X}$: 180.54
										S: 2.62
										$S/\sqrt{n}$: .8715
50% +EDTA	179.3	184.26	191.48	184.66	185.79	183.97	195.6	179.11	193.2	$\bar{X}$: 192.37
										S: 2.05
										$S/\sqrt{n}$: .91(
70% SEW	238.12	235.12	234.16	233.58	238.39	233.7	229.6	229.6	232.79	$\bar{X}$: 232.7
										S: 3.05
										$S/\sqrt{n}$: 1.02
70% +EDTA	240.86	242.89	239.35	243.7	237.63	239.85	255.45	250.06	256.47	$\bar{X}$: 247.45
										S: 7.91
										$S/\sqrt{n}$: 2.9
100% SEW	327.57	319.80	321.93	334.78	333.75	325.91	321.83	319.06	322.83	$\bar{X}$: 326.35
										S: 6.75
										$S/\sqrt{n}$: 2.25
100% +EDTA	316.55	326.27	319.65	333.75	332.72	335.31	317.27	316.21	317.27	$\bar{X}$: 323.71
										S: 8.42
										$S/\sqrt{n}$: 2.81
ANM	144.97	144.22	144.97	152.04	149.64	145.45				$\bar{X}$: 146.79
										S: 3.18
										$S/\sqrt{n}$: 1.06
NON. AUT MILL. R.	3.58	3.55	3.52	4.23	4.41	4.25	4.05	4.05	4.1	$\bar{X}$: 3.99
										S: .348
										$S/\sqrt{n}$: .116

Table A-2. Statistical analysis of EDTA additions; nutrient limitation study of Mill R., 6/24/81.

	MSC mg/l organic $\bar{x}$	Standard deviation s	Standard error $s/\sqrt{n}$	s_1^2/s_2^2	f $\alpha=.995$	$s_1=s_2$?	s_p (pool) YES? s_p^2	t using s_p	t using s_1+s_2	Degrees of Freedom	$t_{.995}$	$\bar{x}_1=\bar{x}_2$?
CONTROL	6.42	0.015	0.006									
+EDTA	7.82	0.234	0.234	.0041	.072, 13.96	NO			-17.89	9.11	3.25	NO
+P	6.81	.020	.007									
+P+EDTA	7.68	0.291	.078	.0047	0.133, 7.50	NO			-8.95	9.09	3.25	NO
+N	29.08	.6956	.222									
+N+EDTA	90.02	.776	.259	.8035	0.133, 7.5	YES	0.543 s_p=.737	-81.49		16	2.921	NO
+N+P	42.66	.949	.313									
+N+P+EDTA	42.23	.932	.311	1.037	0.133, 7.50	YES	.8846 s_p=.940S	0.4695		16	2.921	YES
CONTROL	6.42	0.015	0.006									
+MICRO	7.18	0.168	0.056	0.0079	.072, 13.96	NO			-12.43	9.21	3.25	NO

Table A-3. Statistical analysis of EDTA additions; wastewater additions to Mill River water, 6/24/81.

	MSC (mg/l dry wt) $\bar{x}$	Standard deviation s	Standard error $s/\sqrt{n}$	s^2/s_2^2	f $\alpha = .995$	$\sigma_1 = \sigma_2$?	s_{pooled}^2 YES? s_p^2	t using s_p	t using $s_1 \neq s_2$ NO?	Degrees of Freedom D.F.	$t_{.995}$	$\bar{x}_1 = \bar{x}_2$
+1%	10.24	.126	.042									
+1% + EDTA	11.11	.661	.220	.036	.133 7.50	NO			-3.88	9.653	3.17	NO
+1%	10.24	.126	.042									
PREDICTED	10.03											
+5%	27.0	2.08	0.693									
+5% + EDTA	25.93	0.599	0.20	12.06	.133-7.50	NO			2.566	10.48	3.11	YES
+ 5%	27.0	2.08	0.693									
PREDICTED	18.22											
10%	44.90	1.48	.493									
10% + EDTA	43.97	1.48	.493	1	.133, 7.52	YES	2.1904 $s_p = 1.48$	1.1333		16	2.92	YES
10%	44.90	1.48	.493									
PRED	28.45											
30%	122.47	2.69	0.897									
30% + EDTA	122.32	1.66	0.553	2.63	.133, 7.50	YES	4.996 $s_p = 2.24$	0.1424		16	2.92	YES
30%	122.47	2.69										
PREDICTED	69.39											
50%	180.54	2.62	.8733									
50% + EDTA	182.85	2.73	0.91	.921	.133-7.5	YES	7.159 $s_p = 2.68$	-1.831		16	2.92	YES
50%	180.54	2.62	.87									
PREDICTED	110.33											

Table A-3. continued.

	HSC (mg/Ldry wt) $\bar{x}$	Standard deviation s	Standard error $s/\sqrt{n}$	s_1^2/s_2^2	F $\alpha = .995$	$\bar{x}_1 = \bar{x}_2$	s (POOLED)? YES? s_p^2	using s_p YES? t (using s_p)	NO? t (using $s_1 \neq s_2$)	degrees of freedom D.F.	$t_{.995}$	$\bar{x}_1 \stackrel{?}{=} \bar{x}_2$
70%	233.71	3.06	1.02									
70% +EDTA	245.54	7.41	2.47	0.1706	.1334>7.5	YES	32.14 $s_p = 5.67$	-4.43		16	2.92	NO
70%	233.71	3.06	1.02									
PREDICTED	151.27											
100%	326.36	6.76	2.25									
100% +EDTA	323.72	8.42	2.81	.6931	.133/7.50	YES	55.816 $s_p = 7.47$	0.1003		16	2.92	YES
100%	326.36	6.76										
PREDICTED	212.69											

Table A-4. MSC values for Mill River water sampled 7/16/81; nutrient limitation study and wastewater additions.

	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	
CONTR.	8.14	7.94	8.17	5.08	5.38	5.01	5.29	5.34	5.19	$\bar{x}$ = 6.14 s = 1.44 s/$\sqrt{m}$ = .48
+ P	9.55	9.79	9.79		12.18	12.35	10.0	9.94	10.06	$\bar{x}$ = 10.70 s = 1.29 s/$\sqrt{m}$ = .42
+N	5.26	5.29	5.24	5.44	5.30	5.38	2.70	2.76	2.71	$\bar{x}$ = 4.45 s = 1.3 s/$\sqrt{m}$ = 0.432
+N+P	39.68	39.29	39.39	38.23	36.45	38.38	40.10	39.24	39.24	$\bar{x}$ = 38.90 s/$\sqrt{m}$ = .363 s = 1.09
+EDTA	5.86	5.56	5.66	5.74	5.94	5.74	5.02	5.02	4.77	$\bar{x}$ = 5.50 s = 0.416 s/$\sqrt{m}$ = .139
+EDTA+P	9.13	9.9	9.96	9.68	9.57	9.907	9.73	9.48	9.91	$\bar{x}$ = 9.707 s 0.404 s/$\sqrt{m}$ = 0.135
+EDTA+N	12.86	13.139	13.139	10.47	10.29	10.42	5.14	4.97	4.78	$\bar{x}$ = 9.49 s = 3.56 s/$\sqrt{m}$ = 1.19
+EDTA+N+P	40.36	41.23	40.34	40.65	41.40	41.56	41.66	42.23	41.82	$\bar{x}$ = 41.25 s = 0.67 s/$\sqrt{m}$ = 2233
+MICRO	5.29	5.46	5.25	12.76	12.65	12.718	7.77	8.01	7.83	$\bar{x}$ = 9.65 s = 3.23 s/$\sqrt{m}$ = 1.29
FILT. MILL	3.46	3.54	3.54	0.907	.9262	0.882	2.37	2.36	2.37	$\bar{x}$ = 2.26 s = 1.13
ANH	116.35	119.26	119.76	119.09	125.79	120.22	115.49	110.84	112.45	$\bar{x}$ = 117.71 s = 4.5
5% SEW	33.26	32.34	32.64	32.24	32.01	31.66	34.22	34.11	34.01	$\bar{x}$ = 32.97 s=0.975 s/$\sqrt{m}$ = .325
5% + EDTA	32.41	32.68	32.15	34.96	34.84	35.09	32.56	32.20	32.07	$\bar{x}$ = 33.22 s = 1.32 s/$\sqrt{m}$ = 0.44
5% + MICRO	32.35	30.85	31.75	33.06	31.89	32.01	31.97	31.97	32.08	$\bar{x}$ = 31.99 s = 0.574 s/$\sqrt{m}$ = .192
10% SEW	57.57	58.14	58.14	59.84	59.56	61.376	57.20	56.93	56.93	$\bar{x}$ = 58.41 s = 1.54 s/$\sqrt{m}$ = .513
10% + EDTA	53.12	53.76	52.8	52.22	51.28	51.91	58.14	57.84	59.03	$\bar{x}$ = 54.45 s = 3.01
10% + MICRO	57.54	56.43	57.74	53.47	58.47	57.59	56.91	57.11	56.50	$\bar{x}$ = 57.42 s = 0.742

Table A-4. continued.

	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	
20% SEW	102.68	101.43	102.06	106.8	104.43	106.15	100.79	99.09	99.09	$\bar{x} = 102.45$
										$s = 2.85$
										$s/\sqrt{n} = .95$
20% + EDTA	99.63	101.46	102.06	109.5	108.93	109.5	103.16	102.52	101.43	$\bar{x} = 104.25$
										$s = 3.92$
										$s/\sqrt{n} = 1.31$
20% + HICEO	105.54	104.42	104.42	104.98	107.09	104.98	101.25	105.5	101.25	$\bar{x} = 104.38$
										$s = 1.95$
										$s/\sqrt{n} = 0.65$
50% SEW	202.97	197.54	197.54	201.16	205.18	202.61	215.52	214.04	214.76	$\bar{x} = 205.70$
										$s = 7.24$
										$s/\sqrt{n} = 2.41$
50% + EDTA	217.07	217.83	215.53	240.08	237.8	234.79	228.02	221.64	227.27	$\bar{x} = 227.78$
										$s = 9.22$
										$s/\sqrt{n} = 3.07$
50% + HICEO	236.62	229.05	229.76	224.37	225.85	222.9	209.21	209.96	212.22	$\bar{x} = 222.22$
										$s = 9.674$
										$s/\sqrt{n} = 3.22$

Table A-5. Statistical Analysis of Effect of EDTA&micronutrient additions on nutrient limitation MSC values; Mill R. sampled 7/16/81 ,

	MEAN $\bar{x}$	STANDARD DEVIATION s	STANDARD ERROR $s/\sqrt{n}$	s_1^2/s_2^2	f $\alpha_{.995}$	$s_1 = s_2$?	s_{pooled} YES? s_p^2	YES? $\bar{d}/s_{\bar{d}}$	NO? $\bar{d}$ using $s_{\bar{d}}$	Degrees of Freedom	$t_{.995}$	$\mu_1 = \mu_2$
CONTROL	6.14	1.44	0.48									
CONTROL + EDTA	5.50	0.416	0.139	11.93	.133-7.50	NO			1.28	10.49	3.11	YES
CONTROL	6.14	1.44	0.48									
CONTROL + MICRO	9.65	3.23	1.08	0.199	.133-7.50	YES	5.15 $s_p = 2.27$	-2.13		16	2.92	YES
+ P	10.70	1.29	.43									
+ P + EDTA	9.49	0.404	0.135	10.196	.133-7.5	NO			0.411	10.78	3.11	YES
+ N	4.45	1.3	0.433									
+ N + EDTA	9.49	3.56	1.19	0.1333	.133-7.5	YES	7.13 $s_p = 2.69$	-3.99		16	2.92	NO
+ P + N	38.90	1.09	.3633									
+ P + N + EDTA	41.25	0.67	.2233	2.647		YES	0.7185 $s_p = .905$	-5.51		16	2.92	NO

Table A-6. Statistical analysis of effect of EDTA and micronutrient additions on sewage additions; Mill R. sampled 7/16/81.

	MSC (wet weight) $\bar{x}$	STANDARD Deviation s	Standard error $s/\sqrt{n}$	s_1^2/s_2^2	f $\alpha = .995$	$s_1^2 = s_2^2$	$[s_{pooled}]^2$ YES: s_p^2	YES t (using s_p)	NO t (using $s_1 + s_2$)	Degrees of freedom $= F$	$F_{.995}$	$\bar{x}_1 = \bar{x}_2$?
5% SEW	32.97	.975	.325	0.546		YES	1.347	-0.457		16	2.92	YES
5% + EDTA	33.22	1.32	0.44				$s_p = 1.16$					
5% SEW	32.97	.975	.325	2.865		YES	0.641	-0.662		16	2.92	YES
5% + MICRO	31.99	.576	0.192				$s_p = .801$					
5% SEW	32.97	.975	.325	.119		NO			4.77	11.12		
PRED.	28.22											
10% SEW	58.41	1.54	0.513	0.262		YES	$s_1^2 = 5.72$	3.514		16	2.92	NO
10% + EDTA	54.45	3.01	1.003				$s_p = 2.391$					
10% SEW	58.41	1.54	0.513	4.03		YES	$s_1^2 = 1.18$	1.726		16	2.92	YES
+ MICRO	57.42	0.767	0.256				$s_p = 1.216$					
10% SEW	58.41	1.54	0.53						11.22	9		
PRED.	52.65											
20% SEW	102.45	2.85	.95	0.529		YES	$s_p^2 = 11.74$	-1.114		16	2.92	YES
20% + EDTA	104.25	3.92	1.36				$s_p = 3.427$					
20%	102.45	2.85	0.95	.2436		YES	$s_p^2 = 5.96$	-1.677		16	2.92	YES
20% + MICRO	104.38	1.95	0.65				$s_p = 2.44$					
20%	102.45	2.85	.95									
PREDICTED	101.52											
50%	205.70	7.24	2.41	0.617		YES	$s_p^2 = 68.71$	-5.165		16	2.92	NO
50% + EDTA	227.78	9.22	3.07				$s_p = 8.29$					
50%	205.70	7.24	2.41	0.560		YES	$s_p^2 = 73.00$	-4.10		16	2.92	NO
+ MICRO	222.22	4.674					$s_p = 8.54$					
50%	205.7	7.24	2.41									
PREDICTED	248.13											

Table A-7. MSC values for CES additions to Mill River water sampled 7/16/81.

	Y_1	Y_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	$\bar{X}$	S
CONTROL	10.03	9.69	10.08	9.19	9.00	8.86	7.88	7.67	7.71	8.90	0.957
+EDTA	9.00	8.95	8.48	8.71	8.71	8.62	8.21	8.42	8.16	8.58	0.295
+MICRO	8.98	8.89	9.48	9.63	9.53	9.53	9.18	9.23	9.23	9.30	0.260
ANM	125.46	132.64	124.68	125.44	127.64	125.44	119.48	120.39	121.56	126.97	6.09
5% SEW	20.81	20.72	20.90	20.59	20.59	20.50	20.68	20.77	20.77	20.70	0.126
5% +EDTA	21.72	20.97	20.97	23.14	23.35	23.76	20.56	20.78	21.28	21.84	1.238
5% +MICRO	18.54	18.77	18.54	20.13	19.95	19.95	23.09	23.09	22.31	20.49	1.874
10% SEW	36.66	37.09	36.98	30.51	30.62	29.71	32.76	32.33	32.98	33.29	2.92
+EDTA	42.698	42.98	41.64	31.31	30.41	30.61	43.66	43.46	43.06	38.87	6.10
+MICRO	32.93	31.93	32.63	32.82	32.72	32.82	32.96	32.44	32.44	32.62	0.324
20% SEW	56.875	57.743	55.399	61.463	61.463	62.36	53.836	55.182		58.04	3.303
20% +EDTA	83.359	81.651	82.997	57.424	55.572	56.441	61.462	62.809	63.706	67.27	11.87
20% +MICRO	56.875	53.720	55.399	57.309	56.875	54.979	57.425	57.088	57.425	56.43	1.405
50% SEW	97.72	99.57	96.01	102.32	102.82	102.82	72.51	73.81	72.51	91.12	13.83
50% +EDTA	111.15	111.15	112.45	95.37	99.25	98.47	107.36	106.17	106.57	105.33	6.21
50% +MICRO	95.99	94.01	96.47	83.58	83.58	85.01	68.25	66.86	68.25	82.44	12.09

Table A-8. Statistical analysis of effect of EDTA & micronutrient additions on Mill River water (7/16/81) plus CES.

	MSC (mail dry wt) $\bar{X}$	STANDARD DEVIATION S	STANDARD ERROR $S/\sqrt{M}$	S_1^2/S_2^2	f $\alpha = .995$	$S_1^2 = S_2^2$	$\left[S_{pooled}\right]^2$ YES S_p^2	YES? (USING S_p)	NO t (USING S_1, S_2)	DEGREES OF FREEDOM D.F	$t_{.995}$	$\bar{X}_1 \stackrel{?}{=} \bar{X}_2$
CONTROL	8.90	0.959	0.320									
+EDTA	8.58	0.295	0.0983	10.56	.133 - 7.5	NO			0.958	10.68	3.11	YES
+MICRO	9.30	0.260	0.087	13.67		NO			-1.208	10.32	3.17	YES
PREDICTED	3.78											
5% CSEq	20.70	0.126	0.042									
+EDTA	21.84	1.238	0.413	0.0103		NO			-2.75	9.19	3.25	YES
+MICRO	20.49	1.874	0.624	0.0045		NO			.3354	9.08	3.25	YES
PRED.	13.84											
10% CSEq	33.29	2.92	0.973									
+EDTA	38.87	6.10	2.033	0.229		YES	22.87 $S_p = 4.78$	-2.475		16	2.92	YES
+MICRO	32.63	0.324	0.108	31.23		NO			0.674	9.22	3.25	YES
PRED	23.89											
20% CSEq	58.04	3.303	1.101									
+EDTA	67.27	11.87	3.957	0.065		NO			-2.26	10.17	3.17	YES
+MICRO	56.43	1.405	0.468	4.65		YES	5.57 > 2.362	1.446		16	2.92	YES
PRED	44.02											
50% CSEq	91.12	13.83	4.61									
+EDTA	105.33	6.21	2.07	4.95		YES	114.92 $S_p = 10.72$	-2.81		16	2.92	YES
+MICRO	82.44	12.09	4.03	1.31		YES	168.72 $S_p = 13.00$	1.42		16	2.92	YES
PRED.	104.38											

Table A-9. MSC values for Mill River water sampled 7/16/81 plus lime-treated additions.

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	$\bar{X}$	S
CONTROL	6.557	7.606	6.608	6.926	7.314	8.669	7.116	7.32	7.055	7.085	0.649
5% SEW	27.04	27.955	27.513	28.605	27.291	28.268	30.176	29.709	29.251	28.55	1.02
10% SEW	54.169	52.208	53.279	53.923	53.662	53.662	52.546	52.079	52.631	52.27	0.720
20% SEW	96.673	99.09	97.831	96.673	96.673	96.673	95.909	97.643	97.643	97.21	0.938
5% T. SEW	6.514	6.544	6.447	7.742	7.682	7.227	7.936	7.531	7.622	7.26	0.595
10% T. SEW	8.575	8.291	8.201	13.064	12.668	13.172	12.472	12.755	12.643	11.22	2.23
20% T. SEW										0.48	
5% CSE_q	17.913	18.509	18.022	18.905	19.011	19.164	19.519	18.846	19.192	18.78	0.535
10% CSE_q	29.517	29.757	30.412	32.986	31.939	31.56	31.895	31.437	32.637	31.35	1.215
20% CSE_q	52.533	54.125	53.623	50.617	50.039	50.392	52.649	53.141	53.633	52.31	1.555
5% T CSE_q	9.245	9.348	9.50	9.478	10.024	9.420	13.916	13.681	14.324	10.997	2.251
10% T CSE_q	10.063	10.424	10.154	12.252	11.854	12.031	9.193	9.208	8.404	10.393	1.23
20% T CSE_q	0.684	0.652	0.625	0.235	0.281	0.240	0.294	0.305	0.293	0.401	0.192
ANH	119.86	119.86	119.86	120.41	116.73	120.41	124.00	125.27	124.60	121.23	2.766

Table A-10. MSC values for Mill River water sampled 9/27/81; nutrient limitation study and sewage additions.

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	$\bar{X}$	S.D.
CONTROL	0.298	0.262	0.233	0.441	0.344	0.436	0.303	0.283	0.340	0.338	0.072
+P	2.568	2.729	2.847	2.379	2.504	2.532	3.463	3.455	3.355	2.909	0.421
+N	0.201	0.178	0.167	0.238	0.241	0.207	0.159	0.196	0.232	0.205	0.027
+N +P	28.01	28.25	27.82	29.55	29.93	30.51	31.24	31.09	31.72	29.79	1.48
+EDTA	0.161	0.217	0.197	0.173	0.170	0.168	0.249	0.238	0.219	0.199	0.033
+EDTA+P	2.618	2.588	2.529	2.764	2.797	2.716	2.757	2.837	2.803	2.712	0.108
+EDTA+N	0.189	0.203	0.207	0.246	0.222	0.193	0.241	0.274	0.198	0.219	0.029
+EDTA +N+P	28.19	28.83	27.73	28.52	28.99	29.23	30.72	30.46	30.72	29.29	1.151
+ MICRO	0.235	0.201	0.211	0.237	0.286	0.217	0.293	0.298	0.322	0.267	0.063
NON-AUTO	0.271	0.212	0.172	0.225	0.221	0.188	0.186	0.168	0.266		
5% SEW	34.54	34.33	34.65	36.03	35.92	35.71	37.62	36.06	37.51	35.71	1.25
5% +EDTA	34.43	33.48	34.64	32.92	32.82	32.62	34.54	33.99	33.58	33.67	0.78
10% SEW	47.50	48.76	48.43	62.71	60.98	62.07	63.22	63.83	61.42	57.66	7.13
10% +EDTA	55.75	57.73	57.11	65.30	64.40	65.00	66.79	63.12	66.49	62.41	4.33
15% SEW	94.25	94.57	94.25	100.78	101.37	101.37	98.69	97.20	98.39	97.87	2.99
15% +EDTA	91.02	91.93	92.54	93.15	95.68	93.15	101.00	103.99	101.00	95.94	4.79
25% SEW	114.62	110.80	114.62	155.00	153.46	156.30	146.46	150.44	151.09	139.20	19.62
25% +EDTA	108.90	109.52	109.52	156.01	156.01	156.01	165.33	168.80	151.55	142.41	25.36
50% SEW	228.66	223.38	221.96	239.42	245.91	243.13	271.61	272.35	272.25	246.53	20.87
50% +EDTA	221.06	222.42	219.70	263.80	264.51	265.92	261.22	259.77	259.77	248.69	20.83
ANM	120.12	121.78	120.12	136.21	136.21	136.21	135.34	136.15	135.31	130.83	7.64

Table A-11. Statistical Analysis of effect of EDTA on nutrient additions to Mill R. water sampled 9/27/81.

	MSC (mg/L dry wt) $\bar{x}$	STANDARD Deviation s	Standard error $s/\sqrt{n}$	s_1^2/s_2^2	f $\alpha = .995$	$s_1^2 = s_2^2$	s_{pooled}^2	t (using s_p)	t (use $s_1 \neq s_2$)	Degrees of Freedom D.F.	$t_{.995}$	$\bar{x}_1 = \bar{x}_2$
CONTROL	.539	0.072	0.024	4.76	1135-7.5	YES	$s_p^2 = .00314$ $s_p = .056$	5.265		16	2.92	NO
CONTROL + EDTA	0.199	0.033	0.011									
-)	2.909	0.421	.1403	15.16		NO			1.36	10.18	3.17	YES
TP + EDTA	2.712	0.108	0.036									
+N	0.205	0.027	0.009	0.867		YES	$s_p^2 = .0078$ $s_p = 0.028$	-1.06		16	2.92	YES
+N + EDTA	0.29	0.029	0.0097									
+P +N	29.79	1.48	0.493	1.65		YES	$s_p^2 = 1.758$ $s_p = 1.326$	0.500		16	2.92	YES
+P+N+EDTA	29.29	1.15	0.384									
CONTROL	0.338	0.072	0.024	1.306		YES	0.0045 $s_p = 0.0676$	2.2264		16	2.92	YES
+MICRO	0.267	0.063	0.021									

Table A-12. MSC values for Mill R. water sampled 9/27/81 plus CES additions.

	X_1	Y_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	$\bar{X}$	SD.
CONTROL	0.126	0.113	0.096	0.106	0.127	0.089	0.094	0.126	0.097	0.108	0.017
5% SEW	36.70	36.82	37.83	36.13	36.61	36.25	38.22	37.69	38.68	37.21	0.912
5% + EDTA	37.82	38.06	37.16	40.79	39.56	40.79	37.90	38.26	38.26	38.73	1.325
10%	79.31	77.08	77.08	73.64	73.35	71.27	73.35	71.56	72.45	74.34	2.704
10% + EDTA	73.35	74.54	74.54	74.26	74.26	74.60	70.86	70.86	70.25	73.06	1.847
15%	112.88	116.93	112.03	99.83	108.68	108.68	97.28	95.86	95.58	103.51	8.163
15% + EDTA	114.35	114.62	116.89	117.13	113.73	114.13	110.15	109.67	110.15	113.42	2.832
25%	125.21	128.19	125.32	143.59	143.59	145.88	158.42	157.83	156.64	142.80	13.62
25% + EDTA	167.79	163.33	162.38	161.62	167.15	160.23	164.24	163.95	163.85	163.84	2.428
50%	307.60	305.72	300.09	282.57	274.61	278.59	—	—	—	291.53	14.607
50% + EDTA	281.86	287.62	291.24	298.25	300.93	305.76	303.91	298.74	296.68	294.34	9.017
ANM	156.2	156.82	159.19	159.86	160.47	156.35	156.3	156.3	158.44	157.71	1.759

Table A-13. Statistical analysis of effect of EDTA on Mill R. water sampled 9/27/81 plus nutrient, sewage, and CES additions.

	MSC (mg/L dry wt) $\bar{x}$	STANDARD DEVIATION s	STANDARD ERROR $s/\sqrt{m}$	s_1^2/s_2^2	$\div$ $\alpha=.995$	$s_1^2 \stackrel{?}{=} s_2$	$(s_{pooled})^2$ s_p^2	$\pm$ (using s_p)	t (using s_1+s_2)	Degrees of Freedom D.F	$t_{.995}$	$\bar{x}_1 \stackrel{?}{=} \bar{x}_2$
5% SEW	35.71	1.25	0.449									
+EDTA	33.67	0.78	0.26	2.569	0.133=7.5	YES	1.086 s_p=1.042	4.154		16	2.92	NO
+CSEq.	37.21	0.912	0.304	1.779		YES	1.19 s_p=1.044	-2.91		16	2.92	YES
Pred.	29.31											
5% CSEq	37.21	0.912	0.304									
5% CSEq+EDTA	38.73	1.325	0.4417	2.178		YES	1.294 s_p=1.14	-2.84		16	2.92	YES
PREDICTED	29.31											
10% SEW	57.66	7.13	2.38									
+EDTA	62.41	4.33	1.443	2.711		YES	34.79 s_p=5.899	-1.71		16	2.92	YES
CSEq	74.34	2.804	0.935	6.46		YES	29.35 s_p 5.42	-6.53		16	2.92	NO
PRED	58.04											
10% CEq	74.34	2.804	0.935									
+EDTA	73.06	1.847	0.616	2.305		YES s_1	5.64 s_p=2.37	1.144		16	2.92	YES
PRED	58.04											
15% SEW	97.87	2.99	1.00									
+EDTA	95.94	4.79	1.60	0.3896		YES	15.94 s_p= 3.99	1.03		16	2.92	YES
CSEq	103.51	8.163	2.721	0.1342		YES	37.79 s_p= 6.15	-1.95		16	2.92	YES
PRED	76.76											
15% CEq	103.51	8.163	2.721									
+EDTA	113.42	2.832	0.944	.1204		NO			3.441	11.14	3.11	NO
PRED	86.76											

Table A-13. continued.

	MSC $\bar{x}$	Standard deviation s	Standard Error $s/\sqrt{n}$	s_1^2/s_2^2	$F_{.995}$	$\sigma_1^2=\sigma_2$	$(s_{pooled})^2$ YES? s_p^2	Using s_p^2 t	Using $s_1 \neq s_2$ t	Degrees of Freedom D.F	$t_{.995}$	$\bar{x}_1=\bar{x}_2$
25% SEW	139.20	19.62	6.54									
+ EDTA	142.41	25.36	8.45	0.599	.134.25	YES	510 $s_p=22.67$	-0.300		16	2.92	YES
CSeq	142.8	13.62	4.54	2.08		YES	275.22 $s_p=16.89$	-.452		16	2.92	YES
PRED	144.21	14.42		1.85		YES	295.15 $s_p=17.12$	-.673		16	2.92	YES
25% CSE	142.8	13.62	4.54									
+ EDTA	163.84	2.43	0.809	31.42		NO			-4.56	9.58	3.17	NO
PRED	144.21											
50% SEW	246.53	20.87	6.96									
+ EDTA	248.69	20.83	6.94	1000		YES	434.7 $s_p=20.85$	-0.2198		16	2.92	YES
CSeq	291.53	14.61	2.44	2.04		YES	350.13 $s_p=18.71$	-4.56		13	3.012	NO
PRED	287.84											
50% CSEq	291.53	14.61	2.44									
+ EDTA	293.34	9.017	3.006	2.63		YES	132.13 $s_p=11.49$	-.299		13	3.012	YES
PRED	287.84											

Table A-14. MSC values for alum-treated additions to Mill R. water sampled 9/27/81.

TREAT	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7	Y_8	Y_9	$\bar{Y}$
5% SEW	.1239	.1155	.0904	.1971	.1171	.0850	.1473	.1490	.1629	.1323
5% TS+EDTA	.1222	.1092	.1509	.0803	.0962	.1085	.0919	.1032	.1061	.1075
5% TCSE	.1228	.1094	.0814	.0817	.0791	.0962				.0948
5% TCSE+EDTA	.1275	.0917	.1642	.1173	.1292	.1268	.0951	.1154	.1079	.1128
10% T SEW	.1136	.1030	.1046	.0919	.0856	.1159	.1221	.1127	.1224	.1079
10% TS+EDTA	.1469	.1485	.1219	.0888	.1192	.1088	.1047	.1033	.1342	.1196
10% TSE	.2009	.1420	.1148	.1441	.1488	.1030	.1242	.1437	.1119	.1370
10% TS+EDTA	.1116	.1044	.1040	.1300	.1264	.1153	.1520	.1238	.1230	.1212
15% TSEW	.1370	.1430	.1571	.1334	.133	.1374	.2124	.2312	.2704	.1728
15% TS+EDTA	.1208	.1416	.0922	.1440	.1404	.1343	.1152	.1277	.1203	.1244
15% TCSE	.1696	.1398	.1592	.4482	.3930	.3907	.1934	.1700	.1765	.2489
15% TCSE+EDTA	.1377	.1359	.1366	.1260	.1456	.1326	.1402	.1474	.1366	.1354
25% T SEW	.8855	.1225	.1826	.1037	.1159	.1067	.2066	.1530	.1646	.1501
25% TS+EDTA	.1439	.1413	.1834	.1024	.1248	.1075	.1269	.1209	.1435	.1327
25% TCSE	.2091	.1332	.1573	.1991	.1650	.1570	.2023	.1762	.1621	.1734
25% TCSE+EDTA	.1800	.1550	.1892	.1798	.1780	.1330	.1470	.1431	.1581	.1785
50% T SEW	.2891	.2446	.2195	.1699	.1628	.1585	.1356	.1789	.1408	.1889
50% TS+EDTA	.2128	.2093	.1960	.2052	.1990	.1724	.2436	.1850	.2279	.2062
50% TCS	.6443	.6749	.6575	.7419	.6657	.7078	.7277	.7451	.7306	.6995
50% TCSE+EDTA	.5430	.5421	.5618	.6018	.5829	.5816	.5988	.4515	.4620	.6120
CONTROL	.1148	.1117	.0832	.1163	.0945	.1073	.1028	.1068		.1053
ANM	155.14	154.56	154.56	156.82	155.0	157.44	154.95	157.44	161.10	156.33