Technical Report

Predicting Algal Growth Stimulatory Properties of Treated Wastewater

Daniel J. Wagner
Research Engineer

Michael S. Switzenbaum
Associate Professor of Civil Engineering

August 1987

August, 1987

Env. Eng. Report No. 101-87-9

Technical Report

Predicting Algal Growth Stimulatory Properties of Treated Wastewater

by

Daniel J. Wagner
Research Engineer

and

Michael S. Switzenbaum
Associate Professor

Department of Civil Engineering
Environmental Engineering Program
University of Massachusetts
Amherst, MA 01003

Submitted to the

Massachusetts Department of Environmental Quality Engineering
S. Russell Sylva, Commissioner
Division of Water Pollution Control
Thomas C. McMahon, Director

ACKNOWLEDGEMENT

This study was supported by Research and Demonstration Program funds from the Massachusetts Division of Water Pollution Control (MDWPC: Project Number 83-31). The authors are grateful to Neil M. Ram and Kevin C. Sheehan for help in designing this study and to Richard M. Green and Doris S. Atkinson for assistance in the laboratory. We thank Vance Kaczuwka, Chief Operator of the Ware Wastewater Treatment Plant, for collecting sewage samples.

EXECUTIVE SUMMARY

The nitrogen and phosphorus in wastewater treatment plant effluents promote increased algal growth in natural waters and accelerate eutrophication. Efficient methods of predicting the effect of sewage on receiving waters is important in setting rational nutrient limits. The Algal Assay: Bottle Test (AA:BT) is a promising method for measuring the algal growth stimulatory properties of wastewater directly.

Phosphorus is generally the nutrient of greatest concern in reducing receiving water algal growth. Most of the phosphorus in treated wastewater is orthophosphorus; the form most readily available for plant nutrition, but fortunately the form most easily removed by chemical coagulation and precipitation. Chemical coagulation/precipitation by alum or ferric chloride addition to secondary treatment is the most common technology for wastewater phosphorus removal. Biological processes have also been developed to remove phosphorus.

This report presents a series of algal assays performed on Ware River water and on combinations of Ware Wastewater Treatment Plant (Ware, MA.) effluent and dilution water. The experiments were designed with three objectives: 1) to measure the effect of different levels of of sewage treatment on algal growth, 2) to explore possible limiting nutrient shifts in the Ware River, and 3) to evaluate the reduction in growth stimulation that can be achieved by removing wastewater phosphorus with alum.

Two kinds of experiments were performed in this study: Algal Growth Potential (AGP) tests on upstream Ware River water, and sewage addition experiments with combinations of sewage and dilution water. The AGP experiments included a control treatment to measure productivity level and nutrient spiked treatments to determine limiting nutrient. In the sewage addition experiments, sewage effluent (primary, secondary, and tertiary) was added in four proportions (1, 5, 10, 20%) to two kinds of dilution water (Ware River and distilled). Primary and secondary sewage were collected at the plant. Tertiary sewage was prepared by bench scale alum treatment of secondary sewage at three levels (low alum, medium alum and high alum). All treatment were innoculated with the test algae (Selenastrum capricornutum), incubated for 14 days, and analyzed for Maximum Standing Crop (MSC in mg dry wt./l) by electronic cell counting.

AGP tests showed that both productivity level and limiting nutrient in the Ware river differed between July and October samples. The calculated nitrogen/phosphorus ratio N/P failed to predict the correct limiting nutrient on both dates. The N/P ratios of Ware River water-sewage combinations identified probable conditions for shifts in receiving water limiting nutrient. The limiting nutrient identifications, however, should only be used as a rough guide.

Algal growth potential was highest for primary sewage and lowest for tertiary (alum treated) sewage. Reduction in the concentrations of nitrogen and phosphorus in the sewage accounted for these reductions. Additions of 1, 5, 10, and 20% sewage to dilution water produced a linear increase in maximum standing crop. The linear response held for all types of sewage added to either Ware River water or distilled water. Ware sewage was slightly toxic to algae in high proportions. EDTA and the natural organics in Ware River water ameliorated this toxicity. In general, observed maximum standing crops for sewage-dilution water combinations agreed with predictions based on the measured phosphorus and nitrogen concentrations.

Bench scale wastewater phosphorus removal experiments followed by algal assays gave good estimates of the reduction in algal yield produced by a given alum dose. An aluminum/phosphorus ratio of 1.22 or greater was required to shift Ware River water-sewage combinations from nitrogen to phosphorus limitation. A 96% reduction in ortho-phosphorus concentration by alum precipitation reduced algal growth by 46%.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA:BT - algal assay: bottle test

AGP - algal growth potential

ANM - algal nutrient medium

C - control (dilution water)

E - EDTA chelator addition

M - micronutrient addition

MSC - maximum standing crop

N - nitrogen

P - phosphorus

N/P - nitrogen to phosphorus ratio

INTRODUCTION

The discharge of municipal sewage into natural waters is of environmental concern because it accelerates eutrophication. Eutrophication or nutrient enrichment decreases water quality by causing increased phytoplankton growth and proliferation of aquatic plants. Efficient methods for predicting the algal stimulatory properties of wastewater effluents are important in setting rational limits on wastewater nutrient concentrations. Nitrogen and phosphorus are the nutrients of greatest concern in limiting algal growth in receiving waters.

The Algal Assay Bottle Test (AA:BT) has been frequently cited as a direct method for assessing the algal growth stimulatory properties of wastewater effluents (1-11). In the test's simplest form, a sample of water is inoculated with the standard test algae, <u>Selenastrum capricornutum</u>. After incubation under optimum growth conditions, the biomass of algae produced or Maximum Standing Crop (MSC) is measured. This same test, performed on a mixture of sewage and receiving water, measures the growth stimulatory property of the sewage.

This report analyzes and discusses a series of algal assays performed on Ware River water (Ware River, MA.) and on primary, secondary, and tertiary sewage from the Ware Wastewater Treatment Plant. The experiments were designed to measure the effect of different levels of sewage treatment on algal growth, to explore possible shifts in limiting nutrient in the Ware River, and to evaluate the reduction in growth stimulation that can be achieved by removing wastewater phosphorus with alum. The background section of this report briefly examines sources, forms, and concentrations of wastewater phosphorus; technology for wastewater phosphorus removal, and application of the AA:BT to analysis of wastewater treatment and pollution.

The University of Massachusetts Environmental Engineering Program has produced several reports on application of the AA:BT in wastewater treatment (7-11). This report is the last of that series.

BACKGROUND

Municipal Wastewater Phosphorus

Phosphorus is generally the productivity limiting nutrient in freshwater ecosystems (12). Conventional primary and secondary wastewater treatment plant effluents are major point sources of phosphorus (13). Domestic wastewaters normally contain substantial amounts of phosphorus. Approximately 30-50 percent of the phosphorus in domestic wastewaters originates as human wastes such as feces, urine and waste food. Approximately 50-70 percent originates in detergents containing phosphate builders (14,15). Wastewater treatment plants operating in areas having phosphate detergent bans discharge approximately 50 percent less median annual per capita phosphorus loads than plants in areas without bans (16). Typical phosphorus concentrations in fresh domestic wastewater are: inorganic (4-15 mg P/l), organic (2-5 mg P/l), and total (6-20 mg P/l). Typical phosphorus secondary treatment removal efficiencies are: 10-20% without chemical addition and 80-95% with addition of alum or ferric chloride coagulants (13).

Phosphorus exists in raw wastewater primarily as either organic phosphorus or soluble phosphates. Forms of phosphate include orthophosphate ions, polyphosphates and metaphosphates, which are cyclic polyphosphates (15). Significant amounts of both soluble phosphates and organic phophorus are present in raw wastewater. However, polyphosphates and organic phosphorus can be converted to orthophosphate ions during biological treatment (15). Therefore, a large portion of the phosphorus present in biological effluents is orthophosphate ions. Orthophosphate is the phosphorus form most easily removed by chemical precipitation; unfortunately, it is also the form most readily available for plant nutrition.

Green (11) gives a detailed account of both the soluble and particulate forms of phosphorus and the bioavailability of these forms.

The most common technology for wastewater phosphorus removal is chemical coagulation/precipitation. The most common chemicals used are aluminum and iron salts although lime and polymers are also used (17). The chemicals may be added to either primary sedimentation basins, secondary aeration basins, or as a separate tertiary step. In general, the most widely practiced option is alum or ferric chloride addition to the secondary treatment (17). A more complete description of phosphorus removal by chemical coagulation/precipitation may be found elsewhere (13).

More recently, biological processes have been developed and are being used for phosphorus removal (18). Several different processes exist Phostrip, Bardenpho and the A/O System (19). As of 1984, there were several full scale biological phosphorus removal plants in operation in the United States with more plants in the pilot or development stage (18). However, at present chemical addition remains the process of choice.

Algal Assays for Predicting the Stimulatory Properties of Wastewater

The algal assay: bottle test has been cited quite frequently in the literature as a direct method for assessing the impact of a wastewater loading on the aquatic environment (6,10). Ram (10) noted that the AA:BT has been found to be an effective method for determining:

1. the limiting nutrient of a water,
2. the presence or absence of algal growth inhibitors,
3. possible trace element limitation of a water sample, and
4. the overall productivity level of a water sample.

Ram (10) also noted that the AA:BT may be used as an approach (when the limiting nutrient of the receiving water is known) to predict the effect of wastewater dischargers on the nutrient loading into streams and the resultant algal responses. Previous investigators have used this approach (7,20-24).

The AA:BT can also be used on alum treated wastewater to determine the algal growth response in a water receiving such treated effluent. Results of wastewater AA:BT testing can be used to evaluate (10):

1. the level of improvement in water quality resulting from reduction of wastewater phosphorus loading on the receiving water,

2. the bioavailable phosphorus content in the raw or treated wastewater,

3. any shifts in nutrient limitation below the wastewater outfall which may be attributable to phosphorus removal by alum treatment,

4. any change in trophic status of the receiving water attributable to the alum treatment, and

5. the extent to which established effluent guidelines prevent nutrient enrichment and aquatic plant proliferation in receiving waters.

OBJECTIVES AND APPROACH

This research project had three main objectives: 1) to demonstrate how the AA:BT can measure the growth stimulatory properties of treated sewage, 2) to examine the utility of algal assays in evaluating the level of phosphorus removal necessary to reach some specified goal of reduced algal growth, 3) to show how algal assay data can identify possible shifts in receiving water limiting nutrient from above to below a wastewater discharge.

The Ware Wastewater Treatment Plant sewage and Ware River dilution water were selected for this study. Between August and November 1984, the Ware Plant was upgraded from primary treatment to secondary treatment allowing collection of two distinct sewage types. Secondary effluent was treated with alum in the laboratory to remove phosphorus and produce tertiary treated sewage. Algal assays were performed on combinations of sewage and Ware River water to measure growth and stimulation. The variables chosen for sewage addition experiments along with the rational for their selection are listed in Table 1.

Standard algal growth potential (AGP) tests were performed on Ware River water collected upstream from the plant discharge to determine the background growth potential and limiting nutrient status of the receiving water. Measurements of phosphorus and nitrogen in the sewage and receiving water allowed prediction of the algal yield and of the limiting nutrient in upstream water and in sewage-dilution water combinations.

Table 1. Variables and rational for variable selection for the sewage addition experiments.

VARIABLES	RATIONAL FOR VARIABLE SELECTION
Sewage Type	
-Primary -Secondary -Tertiary	-Test the ability of different levels of sewage treatment to reduce algal growth stimulation.
-Low Alum -Medium Alum -High Alum	-Evaluate the level of phosphorus removal necessary to reach some specified goal of reduced growth stimulation.
Dilution Water Type	
-Ware River water	-Test the sewage from the selected treatment plant in combination with water from the receiving stream. -Check for changes in nutrient limitation status with sewage addition.
-Distilled water	-Distilled water provides a seasonal control for possible changes in receiving water nutrient status.
Sewage Proportions	
-1% -5% -10% -20%	-Choose proportions which bracket combinations of high flow and low flow of plant and receiving stream.
Chelator Addition	
-with EDTA	-Check for possible presence of metal toxicity or micronutrient limitation
-without EDTA	

MATERIALS AND METHODS

Study Site

Sewage samples were collected from the Ware Wastewater Treatment Plant in Ware, MA. The Ware plant was upgraded from primary treatment to extended aeration activated sludge secondary treatment between August and November 1984. Alum coagulation/precipitation for phosphorus removal (tertiary treatment) was added to the plant toward the end of this period. Phosphorus removal takes place in the converted primary plant tank at the end of the treatment process. Design flow for the new plant is 2 mgd (3.1 cfs) with April-October effluent limits of 1.0 mg/l ammonia nitrogen-N and 1.0 mg/l total P.

Waste from the plant is discharged into the Ware River. The average flow (1912-1984) of the Ware River at the U.S.G.S. gauging station at Gibb's Crossing was 280cfs. Thus, at design flow the plant accounts for about 1% of the river's flow on average. Calculation of the plant's contributionat two pertinent low flow conditions are 13% at the 30Q2 of 22.7 cfs and 16% at the 7Q10 of 18.9 cfs (25). The 30Q2 is the 30 day low flow likely to recur every two years. The 7Q10 is the 7 day low flow likely to recur every 10 years.

Sample Collection and Preparation

Primary sewage was collected as a grab sample from the primary clarifier tank effluent weir on 15 July 1984. The primary plant was still operating at this time. Secondary sewage was collected as a 24 hr composite on 6 October 1984 from the collection trough of the final clarifier. The plant was operating without phosphorus removal at this time. Ware River water was collected at mid stream, mid depth just above the Church Street

Bridge in Ware, MA (ca. 3 miles upstream from discharge). All samples were collected in acid washed polyethylene bottles, transported on ice, and refrigerated until experiments began (always less than two days).

Preparation of Ware River water for AGP experiments and as dilution water for sewage addition experiments followed the protocols given in Miller *et al.* (20) and Plotkin and Ram (7). Sewage samples were prepared by sequential filtration through Whatman No. 44 paper, Whatman 934 AH glass fiber filters, and 0.45 µm membrane filters. This procedure agrees with the recommendations for testing sewage given in Miller *et al.* (20).

Algal Growth Potential Experiments

AGP experiments were performed on Ware River water from both collection dates. The protocol followed for these tests is detailed in Miller *et al.* (20) and Plotkin and Ram (7). Briefly, three replicate flasks of each of the solutions listed in Table 2 were innoculated with the test algae (*Selenastrum capricornatum*). The flasks were then incubated under optimal growth conditions for 14 days. The Maximum Standing Crop (MSC, mg dry wt./1) was determined by measuring cell concentration and volume with a Coulter Counter (Model ZBI) and multiplying the numbers by a specific weight coefficient of 3.6×10^{-10} mg/μm^3 (see 7). The Appendix lists the MSC for each replicate flask in the AGP experiments and in the sewage addition experiments described below.

Miller *et al.* (20) and Plotkin and Ram (7) explain AGP test interpretation.

Table 2. Nutrient and chelator additions needed to determine limiting nutrient and possible presence of algal toxicants. Additions were made exactly as described in (12 and 13).

Symbol	Additions to Water
C	Control - Autoclaved Ware River water
P	Control + 0.05 mg P/1
N	Control + 1.00 mg N/1
PN	Control + 0.05 P/1 + 1.00 mg N/1
E	Control + 1.00 mg EDTA/1 (as Disodium Ethylene dinitrilo Tetraacetate)
PE	Control + 0.05 mg P/1 + 1.00 mg EDTA/1
NE	Control + 1.00 mg N/1 + 1.00 mg EDTA/1
PNE	Control + 0.05 mg P/1 + 1.00 mg N/1 + 1.00 mg EDTA/1
M	Control + micronutrients
CU	Unautoclaved Control

*micronutrients are Ca, B, Mn, Fe, Mo, Zn, Cu, Co, S, Mg. Concentrations are exactly as given in (20).

Sewage Addition Experiments

Filtered sewage (primary, secondary and tertiary) was added in four proportions (1, 5, 10 and 20%) to two kinds of dilution water (Ware River and distilled). The metal chelator EDTA was added to an identical set of treatments to detect possible algal toxicants or deficiencies in micronutrients. Distilled dilution water was included as a seasonal control for possible large nutrient changes in the Ware River.

Tertiary sewage was prepared by bench scale alum addition, flocculation, and sedimentation as described in (7) and (26). Alum doses ranging from 20-120 mg alum/l (0.35-2.25 Al/P molar ratio) were added to 500 ml aliquots of secondary sewage. The sewage was rapid mixed for 30 sec at 100 rpm and then slow mixed for 20 min at 20 rpm with a Phipps-Byrd gang stirrer. After one hour of settling, supernatant was filtered as described above. Three of the treated samples were selected for inclusion in sewage addition experiments: one with relatively high residual P (designated Tertiary-Low Alum Dose), one with intermediate residual P (designated Tertiary-Medium Alum Dose), and one with low residual P (designated Tertiary-High Alum Dose).

Sewage-dilution water combinations were inoculated, cultured, and counted exactly as described above for the AGP experiments.

Chemical Analysis and Predicting Algal Yield

Samples of prepared sewage and Ware River dilution water were analyzed for phosphorus and nitrogen according to the methods listed in Table 3. Appropriate reagent blanks and standards were run in all cases.

Predicting the algal yield of a treatment depends on the measured N/P ratio where: N is the Total Soluble Inorganic Nitrogen concentration (TSIN = ammonia-N + nitrite-N + nitrate-N) and P is the soluble reactive phosphorus

Table 3. Methods for measuring water quality parameters.

Parameter	Method	Reference
Ammonia-N	Scaled down colorimetic determination using indophenol reaction	(27)
Nitrate-N	Cadmium Reduction Method	(28)
Nitrite-N	Cadmium Reduction Method	(28)
Orthophosphate	Heteropoly Blue-Ascorbic Acid Spectrophotometric Method	(29)
Total Phosphorus	Potassium persulfate digestion followed by Heteropoly Blue-Ascorbic Acid Spectrophotometric determination	(28,29,30)
pH	potentiometric method	(30)

concentration (commonly called orthophosphorus). Values of N/P less than 11.3 are N limiting while values greater than 11.3 are P limiting (20). For P limiting treatments, the phosphorus concentration (mg P/l) is multiplied by the algal yield factor of 430 to obtain the predicted MSC (mg dry wt. of algae/l). For N limiting treatments, the TSIN concentration (mg N/l) is multiplied by 38 to predict MSC. Failure to attain the predicted MSC may be attributed to micro nutrient limitation, presence of algal toxicants or inaccurate chemical analysis (7,20).

There is an area of nitrogen-phosphorus co-limitation extending at the least between N/P ratios of 10 and 13 (see 7,20). The MSC of samples with co-limitation must still be predicted from either the N or P algal yield factor. Thus, the observed and predicted MSC for co-limiting samples may show poor agreement.

Predicted MSC and the N/P ratio of all experimental treatments are given in the Appendix.

Data Analysis

Miller et al. (20) suggest two methods for analyzing AA:BT data, the first based on percent differences between means and the second based on analysis of variance of the data set in question. The percent difference method, outlined in Table 4 was chosen for this study. The percent difference between treatment means required to assign statistical significance increases as the MSC decreases. This is because small experimental errors inheritantly cause greater variability in measuring small MSC (7).

For reference, the mean and variance of each experimental treatment is given in the Appendix.

Table 4. Criteria for determining the significance of differences between algal assay treatment means. Values are for means of three replicate flasks of test water. Means differing by the specified percent are considered statistically significantly different at the 95% confidence level (after Miller *et al*. 7).

Algal Assay Treatment Means (mg dry wt./1)	Percent difference between treatment means considered significant
MSC less than 1.0	50
MSC between 1.0 and 3.0	30
MSC between 3.0 and 10.0	20
MSC greater than 10.0	10

Quality Assurance/Quality Control

Quality assurance was maintained during this project by adhearing closely to the documentated chemical analysis and AA:BT protocols cited above.

Quality assurance/quality control checks were made both on chemical analysis methods and algal growth results during this study. An EPA water pollution quality control sample was analyzed for phosphorus and nitrogen (Table 5). The values measured in the Environmental Engineering Laboratory of the University of Massachusetts Department of Civil Engineering closely matched the EPA means. A check for glassware contamination and algal culture vigor was performed for both sampling dates (Table 6) by growing algae in replicate flasks of Algal Nutrient Medium (ANM). The values for this study and those measured by Green (16) are quite similar. These values are consistent with ANM yields done in other DCE's Environmental Engineering Algal Assays (7).

Table 5. Analysis of U.S. EPA water pollution quality control sample #4. Measurements were made during routine nutrient analysis between 6 October and 6 November 1984.

Parameter	EPA Recovery Values Mean	 Standard Deviation	 95% Confidence Range	Env. Eng. Lab Values
Ammonia-N (mg/l)	1.90	0.11	1.68-2.12	1.96
Nitrate-N (mg/l)	1.42	0.065	1.29-1.55	1.47
Orthophosphate	0.35	0.01	0.33-0.37	0.35

Table 6. Maximum standing crop of algae grown in standard algal nutrient medium in the UMass Environmental Engineering Laboratory. Samples were run as routine quality assurance checks for culture vigor and glassware contamination. Algal nutrient medium routinely gives yields halfway between the phosphorus concentration prediction (80 mg dry wt./l) and the nitrogen concentration prediction (160 mg dry wt./l)

Dates	Maximum Standing Crop (mg dry wt./l) Number of Samples	 Mean	 Standard Deviation
July 84	3	121	4.1
October 84	3	128	2.4
January-December 84 -from Green (11)	4	116	9.2

RESULTS AND ANALYSIS

Nutrient Concentrations

The Ware River water samples differed considerably in nutrient concentrations on the two collection dates (Table 7). Concentrations of nutrients tended to be higher in July than in October. The N/P ratio was lower for the July sample. Shifts in N/P ratio with season have been noted elsewhere (20).

Nutrient concentrations of sewage samples varied with the type of treatment (Table 7). Primary sewage contained mainly ammonia-N with little nitrate-N or nitrite-N. Nitrification in the extended aeration process converted much of the ammonia to nitrate so, secondary sewage had much more nitrate-N and less ammonia-N. Alum treatment decreased the phosphorus concentration of sewage, but did not alter the concentrations of the nitrogen species.

Algal Growth Potential Experiments

The MSC and limiting nutrient for Ware River water differed on the two collection dates (Fig. 1, 2). The MSC of control flasks was about 40% greater in July than in October (difference significant since 20%, see Table 4). This increased algal yield reflects the increased nutrient levels for the July sample (Table 7).

The observed yields for July indicated phosphorus limitation. Note that the P and PE spiked treatments showed increased growth over the C and E treatments. The N and NE flasks showed no such increase. Toxicity and/or micronutrient limitation for this sample were unlikely since the C, E, and M treatments had essentially equal yields.

Table 7. Chemical data for Ware River water and sewage. River water was autoclaved and filtered before measuring nutrients. Sewage samples were filtered before measuring nutrients.

Sample Type	pH	Total-P	Ortho-P	NO_2-N+ NO_3-N	NH_3-N	Total Soluble Inorganic-N	N/P Ratio
Ware River		µg/l	µg/l	µg/l	µg/l	µg/l	
July 84	7.0	44.6	28.5	246	31	277	9.7
October 84	6.1	28.1	13.3	125	56	181	13.6
Sewage		mg/l	mg/l	mg/l	mg/l	mg/l	
Primary	7.3	6.1	5.4	0.04	22.6	22.6	4.2
Secondary	-	6.5	6.5	5.3	1.0	6.3	1.0
Tertiary Low Alum	-	-	1.3	5.6	1.1	6.7	5.1
Tertiary Med. Alum	-	0.51	0.50	6.2	1.2	7.3	14.7
Tertiary High Alum	-	0.28	0.25	5.9	1.0	6.9	27.6

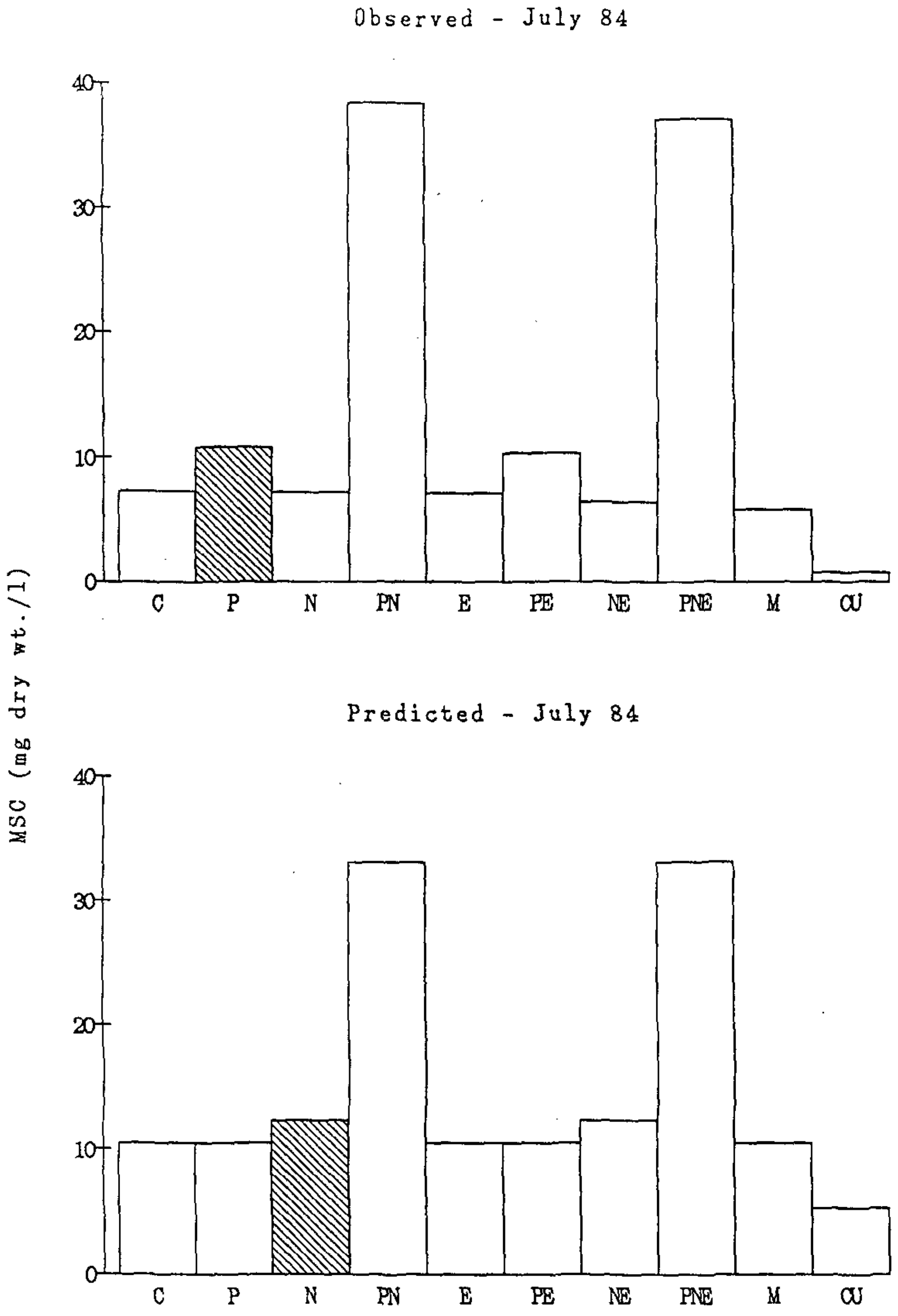

Figure 1. Observed and predicted algal Maximum Standing Crops (MSC) in Ware River water - July 1984. The cross hatched bars identify the observed or predicted limiting nutrient. Letters and letter combinations identify the additions to each treatment: c - control, cu - unautoclaved control, P - phosphorus, N - nitrogen, E - EDTA chelator, M - micronutrients.

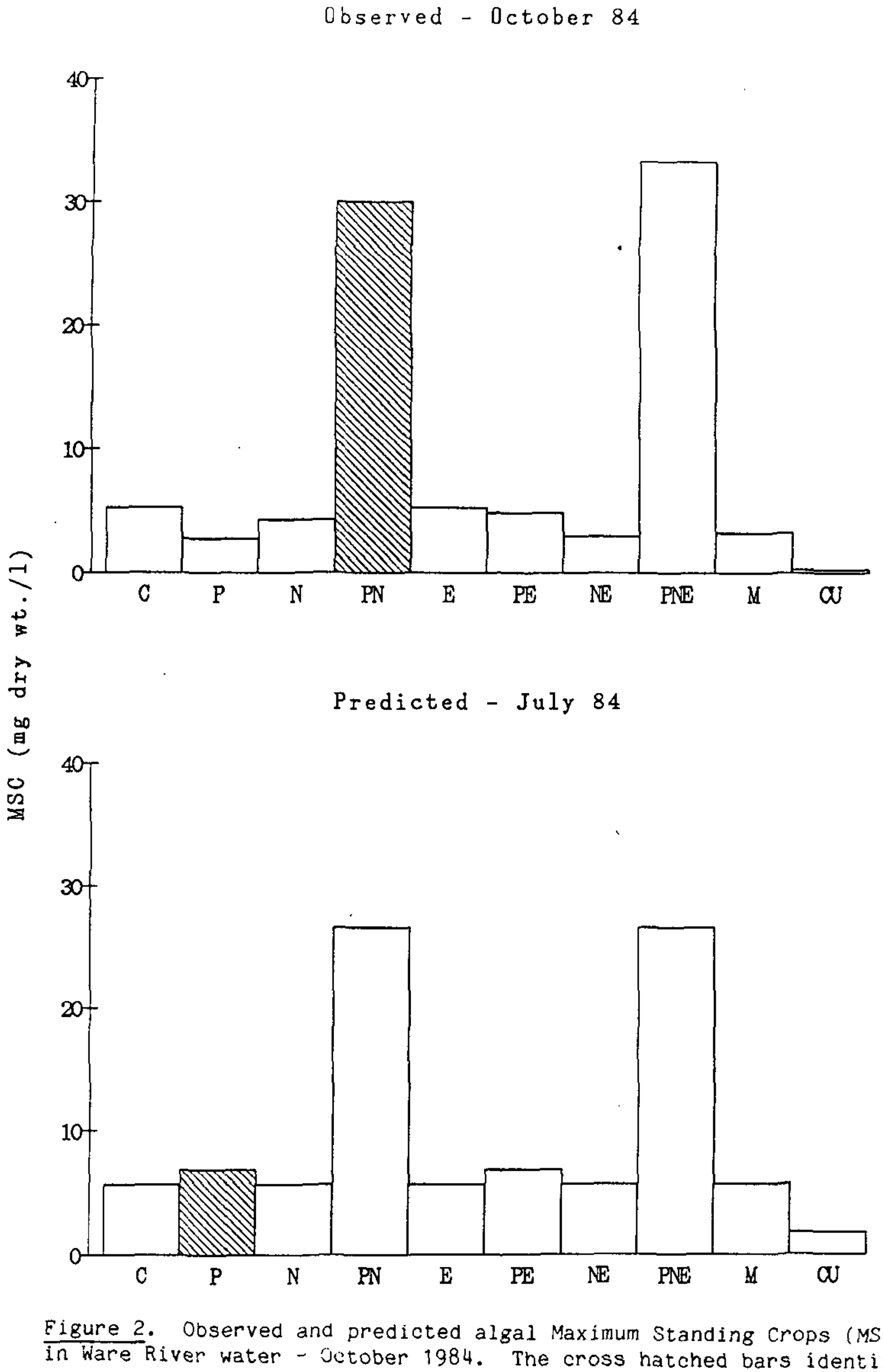

Figure 2. Observed and predicted algal Maximum Standing Crops (MSC) in Ware River water - October 1984. The cross hatched bars identify the observed or predicted limiting nutrient. Letters and letter combinations identify the additions to each treatment: c - control, cu - unautoclaved control, P - phosphorus, N - nitrogen, E - EDTA chelator, M - micronutrients.

The observed and predicted yields for the July sample do not agree. The measured N/P ratio of 9.7 for this sample predicts nitrogen limitation. Thus, the predicted N and NE yield exceeds the predicted control yield. In this case, predicted yields did not match observed yields because the calculated N/P ratio failed to predict the true limiting nutrient. Miller *et al*. (20) state that measured N/P ratios can serve only as approximate guides to nutrient limitation.

AGP results from the October sample were equivocal (Fig. 2). Neither P nor N treatments produced yields consistently greater than control yields. EDTA and micronutrient additions also failed to increase yields. Thus, this sample was most likely nitrogen-phosphorus co-limited. Co-limitation at a N/P ratio of 13.6 is outside the accepted co-limitation range of 10 to 12 for *Selanastrum capricornutum* (20). Chiaudi and Vighi (31) have observed co-limitation at N/P ratios as low as 5.

The 13.6 N/P ratio of the October 1984 Ware River sample demanded yield prediction based on P limitations and the P algal yield factor. Therefore, predicted yields did not match observed yields well.

Analysis of the two Ware River samples demonstrated that predicted yields will inherently show poor agreement with observed yields when:
1) the measured N/P ratio incorrectly predicts the limiting nutrient, and
2) the sample displays nitrogen-phosphorus co-limitation.

Sewage Addition Experiments

Higher levels of sewage treatment decreased the algal stimulatory properties of the sewage (Fig. 3). Primary sewage additions yielded about four times more algae than secondary sewage for all proportions tested. Alum treated sewage yielded less algae than secondary sewage. Reduced

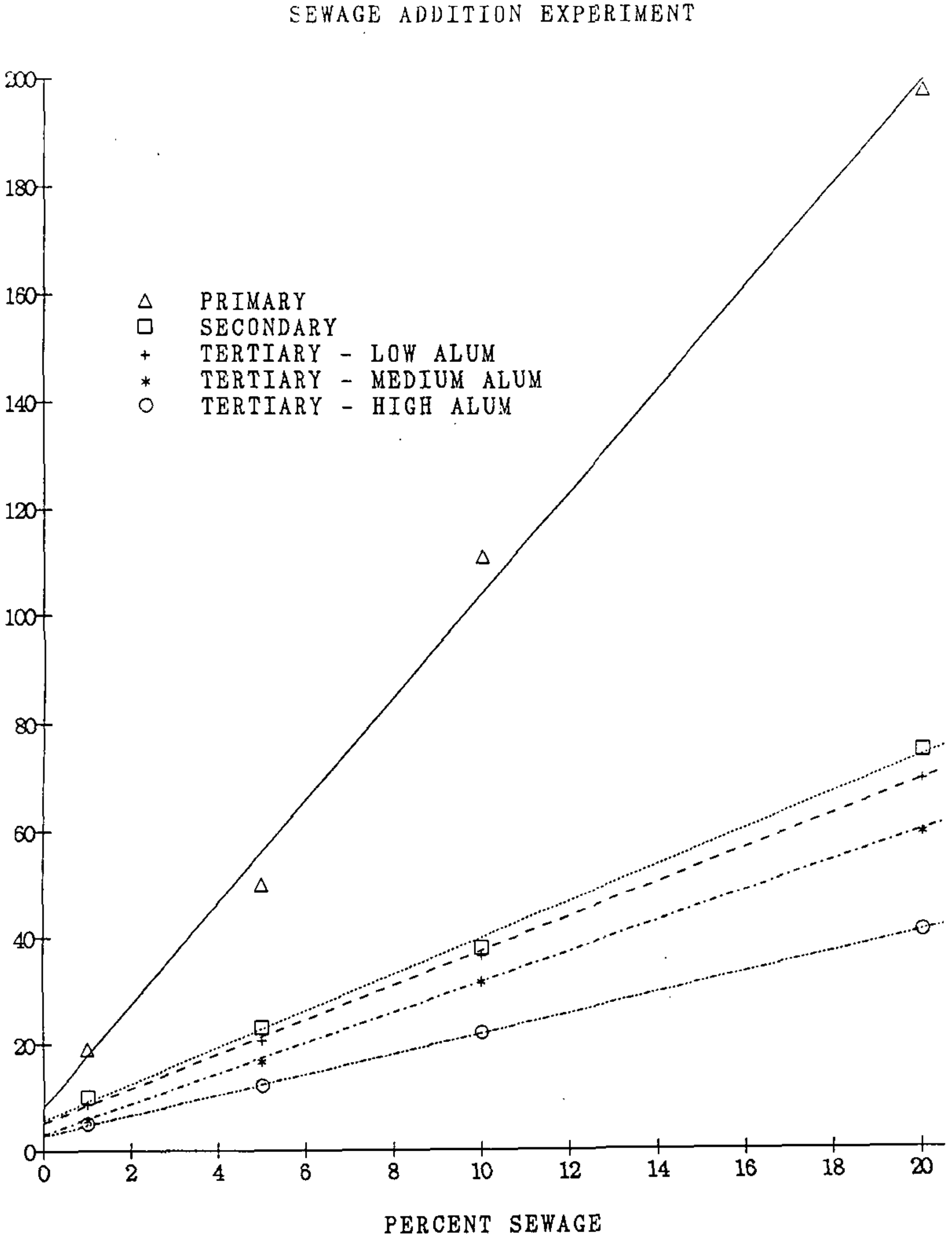

Figure 3. Observed algal Maximum Standing Crops (MSC) in combinations of Ware River water and sewage. Note the linear increase in MSC with increases in sewage proportion and the decrease in MSC with better sewage treatment.

concentrations of nitrogen and phosphorus (Table 7) were responsible for the decreased algal yields.

For all sewage types, there was a linear increase in MSC with increases in sewage proportion (Fig. 3). This same trend was present with distilled dilution water and with EDTA additions to dilution water. Other investigators have reported similar linear trends of increasing algal yield with sewage addition (8-10,21-23).

The mean MSC for all treatments in the sewage addition experiment are presented in Table 8. Several trends are worth noting:

1) Treatment with Ware River dilution water produced higher MSC than treatments with distilled water. Nutrients in the river water account for the differences.

2) Addition of EDTA increased the algal yield over treatments without the chelator. The EDTA may have bound and inactivated some slightly toxic materials in the sewage this promoting more growth.

3) Increase in algal yield due to EDTA was greatest for high sewage additions in distilled water, but less distinct at similar doses in Ware River water. This may indicate that natural organic matter in river water also acts as a chelator of algal toxicants.

Algal yields predicted from measured nutrient concentrations and algal yield factors were generally close to observed values (Table 9). However, observed yields were significantly (> 10%) larger than predicted yields for the higher sewage additions. It is difficult to propose a satisfactory explanation for these differences. It is possible that the algae sequestered organic forms of nitrogen and phosphorus not detected by our analytical methods. Sachdev and Clesceri (32) have demonstrated that organic fractions of wastewater stimulate algal growth.

Table 8. Observed algal maximum standing crops in combinations of dilution water and sewage. Dilution waters were Ware River water and distilled water both with and without EDTA chelator (E) additions.

% Sewage	Dilution Water	Maximum Standing Crop (mg dry wt./l) Primary	Secondary	Tertiary Low Alum	Tertiary Med. Alum	Tertiary High Alum
1	River	19	10	9	6	5
1	River + E	20	10	9	8	7
1	Distilled	0.1	0.1	0.2	0.1	0.1
1	Distilled + E	4	0.1	0.2	0.1	0.1
5	River	50	23	21	17	12
5	River + E	55	23	21	19	15
5	Distilled	30	3	11	3	0.2
5	Distilled + E	43	17	15	5	1
10	River	111	38	37	32	22
10	River + E	111	38	34	34	26
10	Distilled	90	27	28	23	8
10	Distilled + E	94	34	32	22	8
20	River	198	75	70	60	41
20	River + E	194	75	71	61	45
20	Distilled	177	57	62	51	22
20	Distilled + E	183	63	71	55	33

Table 9. Observed and predicted algal maximum standing crops in combinations of Ware River water and sewage.

Sewage Type	% Sewage	Maximum Standing Crop (mg dry wt/l)	
		Observed	Predicted
Primary	1	19	19
	5	50	53
	10	111	95
	20	198	180
Secondary	1	10	9
	5	23	13
	10	38	30
	20	75	53
Tertiary Low Alum	1	9	9
	5	21	19
	10	37	32
	20	70	57
Tertiary Medium Alum	1	6	8
	5	17	15
	10	32	27
	20	60	47
Tertiary High Alum	1	5	7
	5	12	11
	10	22	23
	20	41	26

Most of the sewage-river water combinations had N/P ratios clearly indicative of N or P limitation. However, as with the Ware River samples, it is possible that co-limitation may have occurred outside the generally accepted N/P ratio range or that the N/P ratio predicted the incorrect limiting nutrient and consequently the incorrect algal yield factor.

Limiting Nutrient Shifts

Changes in N/P ratio and limiting nutrient occur with greater degree of sewage treatment and with increasing sewage proportion (Table 10). For all proportions of sewage tested, the medium and high alum doses remove enough phosphorus to shift conditions from N limitation to P limitation. The N/P ratio of Ware River-sewage combinations trend away from the river water ratio and toward the sewage ratio as the sewage proportion increases.

Implications of changing nutrient status in the Ware River below the treatment plant outfall can be made from Table 10. For example, under low river flow conditions (e.g. 10% plant contribution to flow) the phosphorus limiting river water would become nitrogen limiting below the outfall under a secondary treatment scheme. At least a medium alum dose would be required to shift the water below the outfall water to P limitation and reduce the algal growth potential. Because assignment to a limiting nutrient category was based only on calculated N/P ratios (rather than an AGP test) interpretation of nutrient shifts must be treated circumspectly.

Tertiary Treatment Experiment

Increased alum doses decreased the sewage residual P and reduced algal yields in different proportions (Table 11). For example the highest alum dose reduced ortho-P by 96%, but reduced algal growth by only 46%. This is because nitrogen not phosphorus limits growth at the low alum dose (see

Table 10. Limiting nutrient in combinations of Ware River water and sewage based on calculated nitrogen/phosphorus ratios. N = nitrogen. P = phosphorus.

	Nitrogen/Phosphorus Ratio and Limiting Nutrient				
Percent Sewage	Primary	Secondary	Tertiary Low Alum	Tertiary Med. Alum	Tertiary High Alum
0 River Water	9.7 N	13.6 P	13.6 P	13.6 P	13.6 P
1	6.1 N	3.1 N	9.3 N	13.9 P	15.8 P
5	4.7 N	1.0 N	6.5 N	14.2 P	20.6 P
10	4.4 N	1.2 N	5.8 N	14.5 P	23.1 P
20	4.3 N	1.1 N	5.4 N	14.6 P	25.2 P
100 sewage	4.2 N	1.0 N	5.1 N	14.7 P	27.6 P

Table 11. Data on alum dose and phosphorus residual from bench scale coagulation of secondary sewage. The algal growth reduction is averaged over all four combinations of Ware River water and sewage.

	Tertiary Sewage		
	Low Alum	Medium Alum	High Alum
Alum Dose (mg/l)	72	85	93
Residual Ortho-P (mg/l)	1.3	0.50	0.25
Molar Ratio (Al/P)	1.03	1.22	1.33
Algal Growth Reduction (%) as Compared to Secondary Sewage	7	25	46
Ortho-P Reduction (%) as Compared to Secondary Sewage	80	92	96

Table 10). Only after enough P is removed to achieve a N/P ratio of ca. 12 or greater can P removal and growth reduction be proportional. From Table 11 it is possible to estimate the alum dose required to reach some specified level of growth reduction. For example, to achieve a 30% reduction in algal standing crop, an alum dose of about 87 mg/l or 640 lb/mil gal would be required.

The molar ratios of Al/P used in this experiment were lower than typical values (Al/P of 2.1-2.6 for 95% removal, Metcalf and Eddy (33)). The typical values, however, are for full scale operations rather than for bench scale tests. A typical dose at the Ware Wastewater Treatment Plant is 115 mg Alum/l to reduce P concentrations below 2 mg total P/l (Personal communication; Vance Kaczuwka, Chief Plant Operator).

It is important to remember that the AA:BT protocol requires filtering sewage samples to remove indigenous bacteria (20). Filtering removes the particulate phosphorus fraction. This fraction is extremely important in assessing the impact of phosphorus to receiving waters. Several investigators have reported that the majority of phosphorus in wastewaters from activated sludge process plants (with chemical addition to the aeration basin) is in the particulate form (34,35). In terms of practical operation, it is relatively easy to precipitate phosphorus from wastewater but conservative clarifier design is needed to ensure proper removal of the particulate phosphorus (34). About 63% of particulate phosphorus in wastewater can be ultimately algal available (36). Green (11) has proposed a scheme for solubilizing particulate P compatable with the standard AA:BT procedure.

CONCLUSIONS

Three general conclusions from this project pertain directly to the stated objectives:

1) The AA:BT effectively measured the algal growth stimulatory properties of sewage from the Ware Wastewater Treatment Plant. The test gave algal yields for combinations of primary, secondary, or tertiary wastewaters mixed with Ware River receiving water.

2) Bench scale wastewater phosphorus removal experiments followed by algal assays provided good estimates of the reduction in algal yield produced by a given alum dose. Thus, the level of phosphorus removal necessary to reach some specified goal of reduced algal growth stimulation could be estimated.

3) Algal assay data and calculated N/P ratios identified probable conditions for shifts in Ware River limiting nutrient from above to below the wastewater outfall. However, assigning limiting nutrient status from calculated N/P ratios should be interpreted only as a rough guide; the predicted limiting nutrient sometimes fails to match the limiting nutrient identified in an AGP test.

Several more specific conclusions can also be drawn from this study:

4) The algal yield of Ware River water differed by about 40% between the July and October samples. The observed limiting nutrient also differed, i.e., P limitation in July and P-N co-limitation in October. The P/N ratio calculated from chemical analysis failed to predict the correct limiting nutrient on both dates.

5) Algal growth potential was highest for primary sewage and lowest for tertiary (alum treated) sewage. Reduction in the concentrations of nitrogen and phosphorus in the sewage accounted for these reductions.

6) Additions of 1, 5, 10, and 20% sewage to dilution water produced a linear increase in maximum standing crop. The linear response held for all types of sewage added to either Ware River water or distilled water.

7) Ware sewage was slightly toxic to algae in high proportions. EDTA and the natural organics in Ware River water ameliorated this toxicity.

8) In general, observed maximum standing crops for sewage-dilution water combinations agreed with predictions based on the measured phosphorus and nitrogen concentrations.

9) An aluminum/phosphorus ratio of 1.22 or greater was required to shift Ware River water-sewage combinations from nitrogen to phosphorus limitation. A 96% reduction in ortho-phosphorus concentration by alum precipitation reduced algal growth by 46%.

REFERENCES

1. Miller, W., Maloney, T., and Greene, J. (1974) Algal Productivity in 40 Lake Waters as Determined by Algal Assays, Water Res., 8:667.

2. Miller, W., Greene, J., Shiroyama, T., and Merwin, E. (1975) The Use of Algal Assays to Determine Effects of Waste Discharge in the Spokane River System, U.S. Environmental Protection Agency EPA-6001/3-75-0321.

3. Miller, W., and Maloney, T. (1971) Effects of Secondary and Tertiary Wastewater Effluents on Algal Growth in a Lake-River System, Jour. Wat. Pol. Cont. Fed., 43:2361.

4. Maloney, T., Miller, W., and Blind, N. (1973) Use of Algal Assays in Studying Eutrophication Problems, in Advances in Water Pollution Research Sixth Internat. Conf., Jerusalem, S. H. Jenkins (ed.), Pergamon Press.

5. Greene, J., Miller, W., Shiroyama, T., and Maloney, T. (1975) Utilization of Algal Assays to Assess the Effects of Municipal, Industrial, and Agricultural Effluents Upon Phytoplankton Production in the Snake River System, Water Air and Soil Production, 4:415.

6. Maloney, T., Miller, W., and Shiroyama, T. (1972) Algal Responses to Nutrient Additions in Natural Waters: 1. Laboratory Assays, in The Limiting Nutrient Controversy, Special Symposium Vol. 1, American Society of Limnology and Oceanography, 134.

7. Plotkin, S. and Ram, N. M. (1983) Establishment of an Algal Assay Laboratory and Presentation of Several Cases Studies Using AA:BT Data, Report No. Env. Eng. 71-83-2, Dept. of Civil Eng., UMass/Amherst.

8. Ram, N. M. and Plotkin, S. (1983) Assessing Aquatic Productivity in the Housatonic River Using the Algal Assay: Bottle Test, Water Research, 17:1095.

9. Ram, N. M. and Austin, P. E. (1983) Predicting Algal Stimulatory Properties of Wastewater, Jour. of Environmental Engineering (Proc. Am. Soc. Civil Eng.), 109:1099.

10. Ram, N. M. (1983) Algal Assay Methods for Assessing the Impact of Wastewater Effluent on Receiving Waters, Journal of the New England Water Pollution Control Association, 17:10.

11. Green, R. M. (1987) Development of an Alternative Phosphorus-Solubilization Procedure for Algal Assay Wastewater Analysis, Report No. Env. Eng. 93-87-1, Dept. of Civil Eng., UMass/Amherst.

12. Vollenweider, R. A. (1985) Phosphorus, The Key Element in Eutrophication Control, In: Lester, J. N. and Kirk, P. W. W. (Eds), Management Strategies for Phosphorus in the Environment, Selper Ltd, London.

13. Nutrient Control (1983) Manual of Practice FD-7, Facilities Design, Water Pollution Control Federation, Washington, D.C.

14. Task Group Report (1967) Sources of Nitrogen and Phosphorus in Water Supplies, Jour Am Wastewater Association, 59:344.

15. USEPA (1976) Process Design Manual for Phosphorus Removal, Office of Technology Transfer, EPA-430/9-77-013 MCD-37.

16. Gakstatter, J. H., Allum, M. G., Dominquez, S. E. and Crouse, M. R. (1978) A Survey of Phosphorus and Nitrogen Levels in Treated Municipal Wastewater, Jour. Water Pollution Control Fed., 50:718.

17. Switzenbaum, M. S., DePinto, J. V., Young, T. C. and Edzwald, J. K. (1980) A Survey of Phosphorus Removal in Lower Great Lakes Treatment Plants, Jour. Water Pollution Control Fed., 52:2628.

18. Arvin, E. (1985) Biological Removal of Phosphorus from Wastewater, CRC Critical Reviews in Environmental Control, 15:25.

19. Weston, R. (1984) Emerging Technology Assessment of Biological Phosphorus Removal, Prepared for USEPA, Municipal Environmental Research Laboratory, EPA Contract No. 68-03-3055.

20. Miller, W., Greene, J., and Shiroyama, T. (1978) The Selenastrum capricornutum Printz Algal Assay Bottle Test: Experimental Design, Application, and Data Interpretation Protocol, U.S. Environmental Protection Agency, EPA-600/4-68-018.

21. Francisco, D. and Weiss, C. (1973) Algal Response to Detergent Phosphate Levels, Jour. Wat. Pol. Cont. Fed., 45:480-489.

22. Shapiro, J. and Ribeiro, R. (1965) Algal Growth and Sewage Effluent in the Potomic Estuary, Jour. Wat. Pol. Cont. Fed., 37:1034-1043.

23. Ferris, J., Kobayashi, S. and Clesceri, N. (1974) Growth of Selanastrum capricornutum in Natural Water Augumented with Detergent Products in Wastewaters, Wat. Res., 8:1013-1020.

24. Raschke, R. L. and Schultz, D. A. (1987) The Use of Algal Growth Potential Test for Data Assessment, Jour. Water Pollution Control Fed., 59:222.

25. Male, J. W. and Hisashi, O. (1982) Low Flows of Massachusetts Streams, Publication 125, Water Resources Research Center, UMass/Amherst.

26. Martel, C., DiGiano, F., and Pariseau, R. (1974) Pilot Plant Studies of Wastewater Chemical Clarification Using Alum, Report No. Env. E. 44-74-9, Univ. of Mass., Amherst, MA.

27. Ram, N. M. (1979) Nitrogenous Organic Compounds in Aquatic Sources, Ph.D. Thesis, Harvard University, Cambridge, MA, 414 pp.

28. Environmental Protection Agency (1979) *Methods for Chemical Analysis of Water and Wastes*, U.S. Environmental Protection Agency, Cincinnati, Ohio, EPA-600-4-79-020.

29. Strickland, J. D. H. and T. R. Parsons (1972) *A Practical Handbook of Seawater Analysis*, Fisheries Research Board of Canada, Ottawa, Canada, 310 pp.

30. American Public Health Association (1985) *Standard Methods for the Examination of Water and Wastewater*, 16th Edition.

31. Chiaudani, G., and M. Vighi (1976) Comparison of Different Techniques for Detecting Limiting or Surplus Nitrogen in Batch Cultures of *Selanastrum capricornutum*, *Water Res.*, 10:725-729.

32. Sachdev, D. and Clesceri, N. (1978) Effects of Organic Fractions from Secondary Effluent on *Selenastrum capricornutum* (Kutz), Journal of the *Water Pollution Control Federation*, Vol. 50. p. 1810.

33. Metcalf and Eddy, Inc., (1979) *Wastewater Engineering*, McGraw-Hill, NY. 747 pp.

34. Switzenbaum, M. S., DePinto, J. V., Young, T. C. and Edzwald, J. K. (1981) Phosphorus Removal: Field Analysis, *Journal of Environmental Engineering* (Proc. Am. Soc. Civil Eng.), *107*:1171.

35. Black, S. A. (1980) Experience with Phosphorus Removal of Existing Ontario Municipal Wastewater Treatment Plants, In: Loehr, R. C. *et al*. (Eds) *Phosphorus Management Strategies for Lakes*, Ann Arbor Science Publishers, Inc., Ann Arbor.

36. Young, T., DePinto, J., Flint, S., Switzenbaum, M., and Edzwald, J. (1982) Algal Availability of Phosphorus in Municipal Wastewaters, *Journal of the Water Pollution Control Federation*, Vol. 54, No. 11, p. 1505.

APPENDIX

Table A-1. Individual flask maximum standing crops and summary statistics for the 15 July 1984 algal growth potential experiment on Ware River water. Letters and letter combinations identify the additions to each treatment: C-control, CU -unautoclaved control, P-phosphorus, N-nitrogen, E-EDTA chelator, M-micronutrients, ANM-algal nutrient medium.

Algal Growth Potential Experiment 15 July 84
Maximum Standing Crop (mg dry wt./l)

Treatment	Rep 1	Rep 2	Rep 3	Mean	Std. Dev.	Predicted
C	7.15	7.57	7.24	7.32	0.22	10.53
P	10.77	11.00	10.70	10.82	0.16	10.53
N	8.28	5.22	8.18	7.23	1.74	12.26
PN	39.33	38.78	36.67	38.26	1.40	33.11
E	6.86	7.07	7.40	7.11	0.27	10.53
PE	10.16	10.40	10.29	10.28	0.12	10.53
NE	8.06	4.76	6.34	6.39	1.65	12.26
PNE	37.05	37.40	36.60	37.02	0.40	33.11
M	6.03	5.49	5.81	5.78	0.27	10.53
CU	0.77	0.84	0.66	0.76	0.09	5.33
ANM	116.81	120.30	125.05	120.72	4.14	-----

Table A-2. Individual flask maximum standing crops and summary statistics for the 6 October 1984 algal growth potential experiment on Ware River water. Letters and letter combinations identify the additions to each treatment: C-control, CU -unautoclaved control, P-phosphorus, N-nitrogen, E-EDTA chelator, M-micronutrients, ANM-algal nutrient medium.

Algal Growth Potential Experiment 6 October 84
Maximum Standing Crop (mg dry wt./l)

Treatment	Rep 1	Rep 2	Rep 3	Mean	Std. Dev.	Predicted
C	5.29	5.38	5.19	5.29	0.10	5.72
P	3.81	2.05	2.55	2.80	0.91	6.88
N	5.35	3.17	4.26	4.26	1.09	5.72
PN	33.37	24.13	32.14	29.97	5.08	26.57
E	5.31	5.35	5.22	5.29	0.07	5.72
PE	4.44	4.26	6.02	4.91	0.97	6.88
NE	1.48	2.91	4.47	2.95	1.50	5.72
PNE	34.07	32.42	33.35	33.28	0.83	26.57
M	2.75	3.70	3.52	3.32	0.51	5.72
CU	0.22	0.32	0.29	0.28	0.05	1.85
ANM	131.36	126.75	128.01	128.71	2.38	-----

Table A-3. Individual flask maximum standing crops and summary statistics for the 15 July 1984 primary sewage additions. Dilution water type abbreviations are: WR - Ware River, DW - Distilled Water, E - EDTA chelator addition.

		Primary Sewage Addition Maximum Standing Crop (mg dry wt./l)						
Dilution Water Type	Percent Sewage Addition	Rep 1	Rep 2	Rep 3	Mean	Std. Dev.	Predicted	N/P Ratio
WR	0	7.15	7.57	7.24	7.32	0.22	10.53	9.7
WR	1	19.03	18.00	18.54	18.52	0.52	19.04	6.1
WR	5	49.96	49.64	50.39	50.00	0.38	53.01	4.7
WR	10	108.50	109.42	116.00	111.31	4.09	95.49	4.4
WR	20	200.01	207.57	187.32	198.30	10.23	180.50	4.3
WRE	0	6.86	7.07	7.40	7.11	0.27	10.53	9.7
WRE	1	20.53	19.85	19.06	19.81	0.74	19.04	6.1
WRE	5	56.31	56.53	52.81	55.22	2.09	53.01	4.7
WRE	10	108.73	112.60	111.31	110.88	1.97	95.49	4.4
WRE	20	192.50	189.54	199.04	193.69	4.86	180.50	4.3
DW	0	0.08	0.09	0.07	0.08	0.01	------	---
DW	1	0.14	0.07	0.08	0.10	0.04	8.59	4.2
DW	5	34.50	25.49	30.04	30.01	4.51	43.02	4.2
DW	10	88.09	92.49	89.54	90.04	2.24	86.03	4.2
DW	20	176.83	181.49	172.59	176.97	4.45	172.06	4.2
DWE	0	0.07	0.10	0.09	0.09	0.02	------	---
DWE	1	3.94	4.84	4.24	4.34	0.46	8.59	4.2
DWE	5	41.95	43.21	42.45	42.54	0.63	43.02	4.2
DWE	10	94.33	94.22	92.91	93.82	0.79	86.03	4.2
DWE	20	181.97	180.02	187.86	183.28	4.08	172.06	4.2

Table A-4. Individual flask maximum standing crops and summary statistics for the 6 October 1984 secondary sewage additions. Dilution water type abbreviations are: WR - Ware River, DW - Distilled Water, E - EDTA chelator addition.

		Secondary Sewage Addition Maximum Standing Crop (mg dry wt./l)						
Dilution Water Type	Percent Sewage Addition	Rep 1	Rep 2	Rep 3	Mean	Std. Dev.	Predicted	N/P Ratio
WR	0	5.29	5.38	5.19	5.32	0.11	5.72	13.6
WR	1	10.40	9.17	9.97	9.85	0.62	9.20	3.1
WR	5	23.27	23.42	22.91	23.20	0.26	12.77	1.0
WR	10	38.91	39.44	36.86	38.40	1.36	30.10	1.2
WR	20	77.14	75.56	73.30	75.33	1.93	53.28	1.1
WRE	0	5.31	5.35	5.22	5.29	0.07	5.72	13.6
WRE	1	10.18	8.70	9.69	9.52	0.75	9.20	3.1
WRE	5	22.74	23.07	22.13	22.65	0.48	12.77	1.0
WRE	10	38.03	39.22	37.12	38.12	1.03	30.10	1.2
WRE	20	75.77	75.20	73.10	74.69	1.41	53.28	1.1
DW	0	0.08	0.08	0.07	0.08	0.01	-----	----
DW	1	0.10	0.15	0.15	0.13	0.03	2.39	1.0
DW	5	1.72	1.80	1.87	1.80	0.08	11.93	1.0
DW	10	27.00	26.39	26.58	26.66	0.31	23.90	1.0
DW	20	57.01	58.11	56.78	57.30	0.71	47.77	1.0
DWE	0	0.08	0.06	0.05	0.06	0.02	-----	----
DWE	1	0.15	0.07	0.12	0.11	0.04	2.39	1.0
DWE	5	17.18	16.03	17.91	17.04	0.95	11.93	1.0
DWE	10	35.02	33.75	32.99	33.92	1.03	23.90	1.0
DWE	20	66.93	77.50	83.35	75.91	8.32	47.77	1.0

Table A-5. Individual flask maximum standing crops and summary statistics for the 6 October 1984 tertiary sewage additions. Dilution water type abbreviations are: WR - Ware River, DW - Distilled Water, E - EDTA chelator addition.

		Tertiary Sewage Low Alum Dose Addition Maximum Standing Crop (mg dry wt./l)						
Dilution Water Type	Percent Sewage Addition	Rep 1	Rep 2	Rep 3	Mean	Std. Dev.	Predicted	N/P Ratio
WR	1	9.06	8.49	8.27	8.61	0.41	9.34	9.3
WR	5	20.77	21.14	20.80	20.90	0.21	6.46	6.5
WR	10	35.06	35.45	39.38	36.63	2.39	31.73	5.8
WR	20	69.57	68.88	70.32	69.59	0.72	56.58	5.4
WRE	1	9.06	9.42	8.46	8.98	0.49	9.34	9.3
WRE	5	22.06	21.54	19.41	21.00	1.40	6.46	6.5
WRE	10	35.55	31.86	33.93	33.78	1.85	31.73	5.8
WRE	20	70.88	72.18	70.88	71.31	0.75	56.58	5.4
DW	1	0.31	0.12	0.15	0.19	0.10	2.55	5.1
DW	5	11.21	11.23	11.23	11.22	0.01	12.77	5.1
DW	10	27.66	28.57	28.13	28.12	0.46	25.54	5.1
DW	20	62.93	60.61	63.05	62.20	1.38	51.07	5.1
DWE	1	0.12	0.09	0.26	0.16	0.09	2.55	5.1
DWE	5	14.38	16.23	15.26	15.29	0.93	12.77	5.1
DWE	10	32.41	32.60	31.55	32.19	0.56	25.54	5.1
DWE	20	71.17	73.32	67.96	70.82	2.70	51.07	5.1

Table A-6. Individual flask maximum standing crops and summary statistics for the 6 October 1984 tertiary sewage medium alum dose addition. Dilution water type abbreviations are: WR - Ware River, DW - Distilled Water, E - EDTA chelator addition.

		Tertiary Sewage Medium Alum Dose Addition Maximum Standing Crop (mg dry wt./l)						
Dilution Water Type	Percent Sewage Addition	Rep 1	Rep 2	Rep 3	Mean	Std. Dev.	Predicted	N/P Ratio
WR	1	6.08	6.25	5.46	5.93	0.42	7.83	13.9
WR	5	16.58	19.33	16.27	17.39	1.68	15.12	14.2
WR	10	31.39	33.45	31.68	32.17	1.12	26.62	14.5
WR	20	59.82	60.61	59.03	59.82	0.79	47.30	14.6
WRE	1	8.17	9.32	7.04	8.18	1.14	7.83	13.9
WRE	5	19.42	19.76	18.62	19.23	0.59	15.12	14.2
WRE	10	32.78	34.64	33.25	33.56	0.97	26.62	14.5
WRE	20	62.25	59.40	61.47	61.04	1.47	47.30	14.6
DW	1	0.12	0.03	0.05	0.07	0.05	2.15	14.7
DW	5	1.35	3.88	5.22	3.48	1.97	10.75	14.7
DW	10	23.36	23.27	22.66	23.10	0.38	21.46	14.7
DW	20	52.50	49.15	50.87	50.84	1.68	42.91	14.7
DWE	1	0.03	0.05	0.06	0.05	0.02	2.15	14.7
DWE	5	4.92	6.83	3.90	5.04	1.20	10.75	14.7
DWE	10	23.33	22.91	19.75	22.00	1.96	21.46	14.7
DWE	20	57.28	51.04	58.04	55.45	3.84	42.91	14.7

Table A-7. Individual flask maximum standing crops and summary statistics for the 6 October 1984 tertiary sewage high alum dose addition. Dilution water type abbreviations are: WR - Ware River, DW - Distilled Water, E - EDTA chelator addition.

Dilution Water Type	Percent Sewage Addition	Tertiary Sewage High Alum Dose Addition Maximum Standing Crop (mg dry wt./l)						
		Rep 1	Rep 2	Rep 3	Mean	Std. Dev.	Predicted	N/P Ratio
WR	1	4.96	5.15	5.18	5.10	0.12	6.75	15.8
WR	5	12.02	9.70	13.19	11.64	1.78	10.79	20.6
WR	10	21.66	22.19	21.94	21.93	0.27	15.91	23.1
WR	20	42.61	41.62	39.32	41.18	1.69	26.06	25.2
WRE	1	6.66	7.63	6.87	7.05	0.51	6.75	15.8
WRE	5	15.18	15.87	13.54	14.86	1.20	10.79	20.6
WRE	10	26.46	25.46	26.48	26.13	0.58	15.91	23.1
WRE	20	49.35	43.92	41.79	45.02	3.89	26.06	25.2
DW	1	0.05	0.06	0.04	0.05	0.01	1.08	27.6
DW	5	0.30	0.16	0.29	0.25	0.08	5.38	27.6
DW	10	6.87	8.18	8.23	7.76	0.77	10.75	27.6
DW	20	23.23	21.03	20.37	21.54	1.50	21.50	27.6
DWE	1	0.09	0.09	0.06	0.08	0.02	1.08	27.6
DWE	5	0.80	1.29	1.03	1.04	0.25	5.38	27.6
DWE	10	7.03	7.41	8.52	7.65	0.77	10.75	27.6
DWE	20	32.86	32.85	32.31	32.67	0.31	21.50	27.6