REC-ERC-80-1

# EVALUATION OF THE ALGAL ASSAY BOTTLE TEST

**Engineering and Research Center**
**Water and Power Resources Service**

**September 1979**

MS-230 (3-78)
Bureau of Reclamation

# TECHNICAL REPORT STANDARD TITLE PAGE

| 1. REPORT NO. | 2. GOVERNMENT ACCESSION NO. | 3. RECIPIENT'S CATALOG NO. |
|---|---|---|
| REC-ERC-80-1 | | |

| 4. TITLE AND SUBTITLE | 5. REPORT DATE |
|---|---|
| Evaluation of the Algal Assay Bottle Test | September 1979 |
| | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) | 8. PERFORMING ORGANIZATION REPORT NO. |
| Richard A. Roline and Victor S. Miyahara | REC-ERC-80-1 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS | 10. WORK UNIT NO. |
| Engineering and Research Center<br>Water and Power Resources Service<br>Denver, Colorado 80225 | 11. CONTRACT OR GRANT NO. |
| | 13. TYPE OF REPORT AND PERIOD COVERED |
| 12. SPONSORING AGENCY NAME AND ADDRESS | |
| Same | 14. SPONSORING AGENCY CODE |

15. SUPPLEMENTARY NOTES


16. ABSTRACT

The algal assay bottle test has been used in the study of water quality and nutrient enrichment on many Water and Power projects. Water samples from 12 sites in the Western United States were used in this evaluation of the test. Actual cell counts and dry mass measurements were the best indicators of algal production. In most studies, the growth of the test alga *Selenastrum capricornutum* Printz responded positively to an increase in nutrient concentration; however, the selected test alga may not be the best test organism for all fresh waters. Some inhibition of growth may have been due to high salinity or toxic elements such as heavy metals or herbicides in the water systems. Predictions of water quality in reservoir planning can be made using AABT results; however, since the test is still not totally conclusive, predictions should be judicious.



17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS-- / algal assay/ reservoirs/ chemical properties/ inhibition/ algal growth potential/ particle counting/ biomass/ reservoir planning/ source water

b. IDENTIFIERS-- / Surface waters of Arizona, Wyoming, Colorado, and South Dakota

c. COSATI Field/Group 06F COWRR: 0601


| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS (THIS REPORT) | 21. NO. OF PAGES |
|---|---|---|
| *Available from the National Technical Information Service, Operations Division, Springfield, Virginia 22161.* | UNCLASSIFIED | 28 |
| | 20. SECURITY CLASS (THIS PAGE) UNCLASSIFIED | 22. PRICE |

GPO 845-630

REC-ERC-80-1

# EVALUATION OF THE ALGAL ASSAY BOTTLE TEST

by

**Richard A. Roline**
**Victor S. Miyahara**

**September 1979**

Applied Sciences Branch
Division of Research
Engineering and Research Center
Denver, Colorado

SI METRIC

**UNITED STATES DEPARTMENT OF THE INTERIOR ★ WATER AND POWER RESOURCES SERVICE**

## ACKNOWLEDGMENTS

Funding and support for this evaluation were provided under DR-415 and by various Water and Power Resources Service regional and project offices. The Chemistry, Petrography, and Chemical Engineering Section performed most of the chemical analyses.

On November 6, 1979, the Bureau of Reclamation was renamed the Water and Power Resources Service in the U.S. Department of the Interior. The new name more closely identifies the agency with its principal functions—supplying water and power.

CONTENTS

TABLES

FIGURES

## ABBREVIATIONS AND SYMBOLS

| | |
|---|---|
| AABT | algal assay bottle test |
| AGP | algal growth potential |
| ATP | adenosine triphosphate |
| BOD | biological oxygen demand |
| EPA | Environmental Protection Agency |
| mg/L | milligrams per liter |
| $\mu$g/L | micrograms per liter |
| $\mu$S/cm | microsiemens per centimeter |
| pH | hydrogen-ion coefficient |
| SDI | standard diversity index |
| TDS | total dissolved solids |
| B | boron |
| C | carbon |
| Ca | calcium |
| Cl | chlorine |
| Co | cobalt |
| $CO_2$ | carbon dioxide |
| Cu | copper |
| Fe | iron |
| H | hydrogen |
| HCl | hydrochloric acid |
| $HCO_3$ | bicarbonate |
| K | potassium |
| Mg | magnesium |
| Mn | manganese |
| Mo | molybdenum |
| N | nitrogen |
| Na | sodium |
| $Na_2$ EDTA | disodium ethylenedinitrilo tetraacetate |
| $NO_3$ | nitrate |
| O | oxygen |
| P | phosphorus |
| $PO_4$ | ortho-phosphate |
| $SO_4$ | sulfate |
| Zn | zinc |

# INTRODUCTION

Eutrophication is a process by which bodies of water become enriched through the natural or artificial addition of nutrients and the effects caused by the addition of these nutrients [1][1].

In recent years, eutrophication of surface waters has been greatly accelerated due to the ever increasing demand on the environment. The increasing use of natural water systems for municipal and industrial waste treatment and removal, and the extensive use of agricultural fertilizers in watershed areas are excellent examples of this increasing environmental pressure.

As water bodies become more productive through the eutrophication process, many problems begin to appear. Drinking water may impart an offensive odor and taste due to waste products produced by algae "blooms," swimming beaches may be closed because of an increase in aquatic pest populations, and beneficial aquatic organisms may be killed or driven from the area by the lowering or elimination of the dissolved oxygen in the water through a high BOD (biological oxygen demand) or algal respiration [2]. These reasons and more make it imperative that we understand the eutrophication process so that we are able to reduce or eliminate the input into this phenomenon.

To be able to judge natural and artificial contributions to a water body's nutrient load and accurately assess the stage of enrichment of various natural waters, many measurements can and must be used. Biological assessment may involve the identification and population densities of fish, aquatic invertebrates, and plankton, as well as the biomass of aquatic macrophytes. Chemical measurements of water quality include analyses for general water chemistry, nutrients, or toxic elements such as heavy metals and herbicides. Physical parameters usually measured are pH, temperature, conductivity, dissolved oxygen, and oxidation-reduction potential. Many of these techniques should be used in combination to be able to accurately determine present levels of enrichment or predict future eutrophication problems.

One of the biological "tools" for measuring nutrient levels in surface waters is the AABT (algal assay bottle test). According to Liebig's "Law of the Minimum," only the addition of the limiting nutrient or the elimination of the limiting condition will cause an increase in algae growth. The AABT uses the growth response of test algae to determine the levels of available nutrients in a water sample as it compares to a control nutrient series. This test may be used to

(a) assess a receiving water to determine its sensitivity to change and its present status,
(b) evaluate materials and products to determine their potential effects on algal growth in receiving waters,
(c) assess the effects of changes in waste treatment processes on algal growth in receiving waters, and
(d) assess the impact of nutrients in tributary waters on algal growth in lakes and receiving waters [3].

The concept of using algal growth response to determine nutrient levels in natural waters has been used for many years. In 1968, the Joint Industry/Government Task Force on Eutrophication met to begin standardization of the AABT. In 1971, the EPA (Environmental Protection Agency), with the assistance of eight government, private, and university laboratories, established a basic protocol to standardize the AABT using the unicellular green alga *Selenastrum capricornutum* Printz as the test organism [4]. The EPA has recently summarized the research to standardize the experimental design, application, and data interpretation of the AABT [5].

In this report, the AABT technique will be evaluated using selected test data from 12 water sources from Arizona, Wyoming, Colorado, and South Dakota. Inconsistencies and problems with the technique will be pointed out and examined.

# APPLICATION

The AABT was originally designed to evaluate point source pollution and the effects on rivers and lakes. Water and Power has the need to be able to judge the nutrient levels of its project waters or to be able to assess the impact of proposed construction on water quality. This knowledge will help designers and planners of canal and reservoir systems to better understand water quality and potential eutrophication problems.

[1] Numbers in brackets refer to entries in the bibliography.

## SUMMARY

- In most fresh waters, the test alga *Selenastrum capricornutum* Printz will respond positively to an increase in nutrient concentration.

- Available nutrient levels for algal growth in the natural water system can be interpolated from growth response comparison of the test waters to the control nutrient series.

- Limiting nutrients and the degree of inhibition or toxicity of certain elements to algae production may be identified and evaluated.

- *Selenastrum carpicornutum* Printz may not be the best test algae for use in all fresh waters, but usually responds favorably.

- Varying environmental conditions and geographic locations of the test waters, as well as constantly changing physical and chemical water parameters, makes predicting algae problems using only the AABT extremely difficult.

## METHODS AND MATERIALS

The water to be tested must be sampled using a clean, acid-washed glass or autoclavable, nontoxic plastic container. A 10 percent by volume HCl acid solution is used to clean the containers to assure removal of toxic elements or any traces of soap containing nutrients. This container is used to sample the water directly and should be dipped about 0.3 m below the surface of the water to avoid collecting surface film and debris. Various depths may be sampled to account for differences in water chemistry due to stratification. The water sample must be refrigerated at $<4$ °C and kept in the dark.

The standard nutrient solution is made from various salts, and in the concentrations shown in table 1.

These nutrient solutions may be prepared to 1000 times the final concentrations needed. Therefore, the test waters are spiked with 1.0 mL of each nutrient solution per liter of water to obtain a 1.0X standard nutrient addition. The nutrients are added to 500-mL Pyrex Erlenmeyer flasks containing 250 mL of the test and control waters[2] in proportions of 0.03X,

[2] Distilled water is used in the control nutrient series.

Table 1.—*Composition of standard nutrient solution for AABT test*[1]

| Compound | Concentration | Element | Concentration |
|---|---|---|---|
| $NaNO_3$ | 25.500 mg/L | N | 4.200 mg/L |
| $K_2HPO_4$ | 1.044 | K | 0.469 |
| | | P | 0.186 |
| $MgCl_2 \cdot 6H_2O$ | 12.164 | Mg | 2.904 |
| $MgSO_4 \cdot 7H_2O$ | 14.700 | S | 1.911 |
| $CaCl_2 \cdot 2H_2O$ | 4.410 | Ca | 1.202 |
| $NaHCO_3$ | 15.000 | Na | 11.001 |
| | | C | 2.143 |
| $H_3BO_3$ | 185.520 µg/L | B | 32.460 µg/L |
| $MnCl_2 \cdot 4H_2O$ | 415.610 | Mn | 115.374 |
| $ZnCl_2 \cdot 4H_2O$ | 3.271 | Zn | 1.570 |
| $CoCl_2 \cdot 6H_2O$ | 1.428 | Co | 0.354 |
| $CuCl_2 \cdot 2H_2O$ | 0.012 | Cu | 0.004 |
| $Na_2MoO_4 \cdot 2H_2O$ | 7.260 | Mo | 2.878 |
| $FeCl_3 \cdot 6H_2O$ | 160.000 | Fe | 33.051 |
| $Na_2EDTA \cdot 2H_2O$ | 300.000 | - | - |

[1] The protocol for this test is based on the procedure described in "The *Selenastrum Capricornutum* Printz Algal Assay Bottle Test" [5].

0.3X, 1.0X, and 3.3X of the standard nutrient addition.

All test waters are then sterilized by autoclaving and/or filtration to remove any endemic species of algae, which will allow testing with a pure population of the test alga.

After the sterilization of waters and stabilization of temperature to 24 °C (±2 °C), the test waters are inoculated with approximately 1000 cells per milliliter of the test alga *Selenastrum capricornutum* Printz. This species of unicellular green algae has been selected by EPA as the test organism because of its unicellular nature which allows for easier cell counting on electronic particle counters and its wide range of adaptability to various water types.

The test flasks are capped with sterilized foam or cotton plugs and immediately incubated in a constantly lighted growth chamber (fig. 1) at the previously mentioned temperature. Illumination in the chamber is provided by fluorescent bulbs to allow 4306±215 lux at the liquid level of the culture flasks. The flasks should be shaken constantly at 100 oscillations per minute or hand-swirled daily for a period of 21 days.

At various intervals throughout the test period, for example at 7, 14, and 21 days, the algal growth must be measured and recorded for all flasks. At least three replications are used at each nutrient concentration level. Algal growth response has been measured by a number of procedures including ATP concentrations, pH changes, total carbon analysis, chlorophyll extraction or autofluorescence, cell counts, and dry mass measurements. Our laboratory uses cell counts, pH measurements, and dry mass to determine algae production. Cell counting is performed using the model ZBI Coulter Counter (fig. 2), pH is measured using the Beckman Electromate digital pH meter, and dry mass is measured according to Stein [6]. Shoaf and Lium [7] evaluated the electronic particle counter method in measuring algal growth and found it to be quite satisfactory.

## RESULTS

The following paragraphs discuss comparisons that are based on the results of a number of tests performed using the AABT method on water samples from 12 sources in the Western United States from the spring of 1976 through the summer of 1979. Algal assay tests were performed on waters ranging from a very high quality at Twin Lakes, Colo., to very eutrophic at the South Platte River in Colorado. We also studied four water samples from the Central Arizona Project that contained various levels of salinity, and other water sources that were between the two extremes in productivity. Additional data from studies not specifically discussed in the results are included in the appendixes for comparative purposes by the reader.

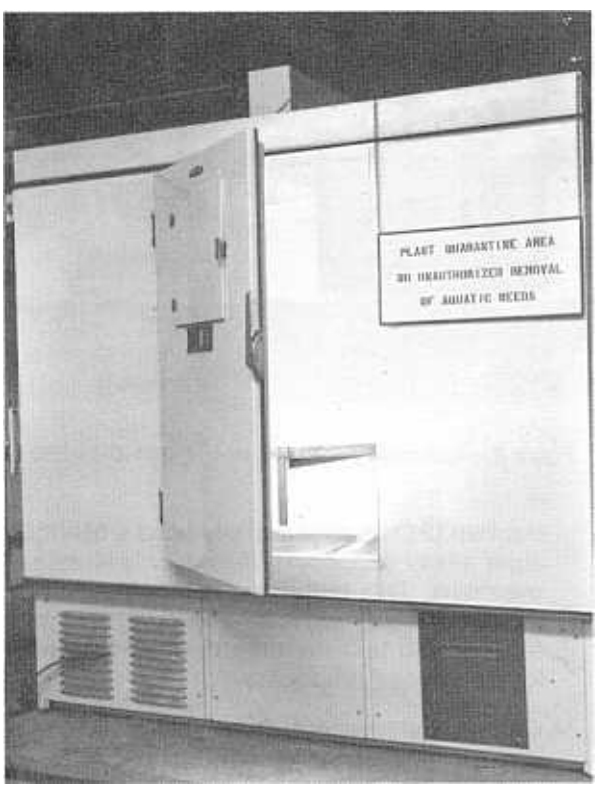

Figure 1.—Constant light and temperature growth chamber. P-801-D-79389.

- The measurement of algal production through a rise in pH in the test waters is a good indication of algal growth over a short period of time. This pH change is caused by the absorption of $CO_2$ by the test algae. The rise in pH has been shown (fig. 3) to reflect algal growth and to be quite reliable up to about the seventh day of the test period. After this time, the pH will begin to drop due, in part, to the reabsorption of $CO_2$ from the air by the test waters [8].


Figure 2.—Electronic particle counter. P-801-D-79390.

Hannan [9] has successfully used a 60-hour algal assay procedure based on pH measurements. This technique has been used to measure algal growth potential, growth rate, and to test the effects of various toxicants on algal production.

- Chlorophyll concentrations have been examined with varying results (apps. A, B, C, and D). The chlorophyll measurements do not seem to be a reliable indication of algal production at the end of the 21-day test period because much of the algae has entered senescence and the chlorophyll is lost. The concentration of the pheo-pigments, degradation products of chlorophyll, have been examined, but as yet we are unable to obtain a direct relationship between the loss of chlorophyll with a gain in pheo-pigment concentration.

- Cell counts at 21 days correlate well with the 21-day dry mass measurements (fig. 4). In some instances, the dry mass measurement may indicate more algal production

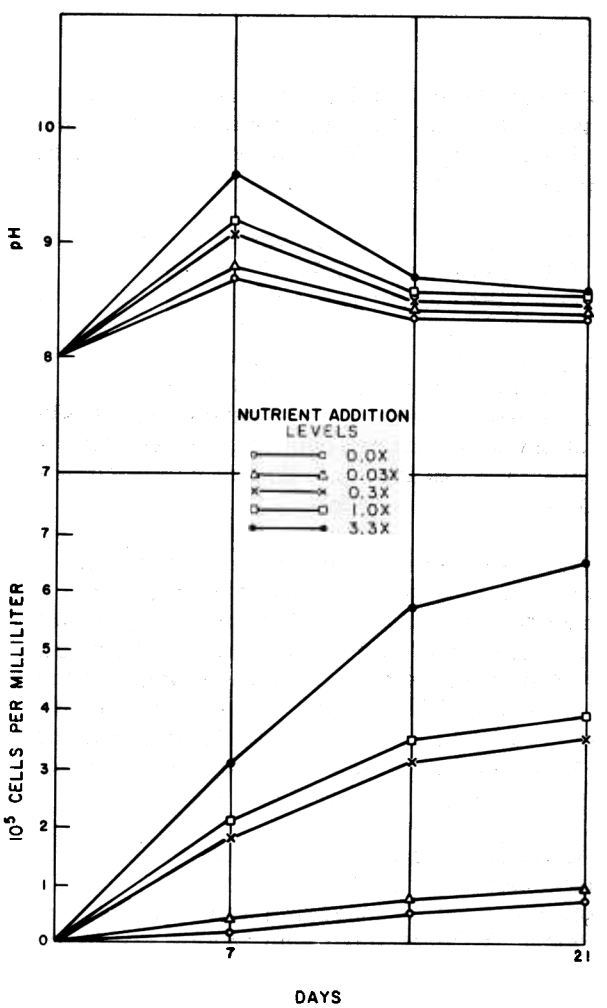


Figure 3.—Comparison of cell counts to pH values. Mean values of eight AGP tests.

than shown by the electronic cell counting method. This is probably because of clumps formed by the test algae which are not counted in the particle counting procedure. Sonification of the sample for 1 minute has partially relieved this problem, but extreme care must be taken to not disrupt the cell membranes of the algae in the water sample. An extraordinarily high dry mass value may result from the presence of silt or debris in an unfiltered water sample and may be corrected by comparisons with the cell counts and pH values. This stresses the fact that it is extremely important to have more than a single indicator of algal production.

- A method for determining limiting nutrients in a natural water system has been examined on water samples from Twin Lakes, Colo. These lakes are two high-mountain lakes in an oligotrophic status. The levels of total phosphorus and total Kjeldahl nitrogen in these waters are 0.003 and 0.054 mg/L in the lower lake and <0.001 and 0.040 mg/L in the upper lake, respectively.

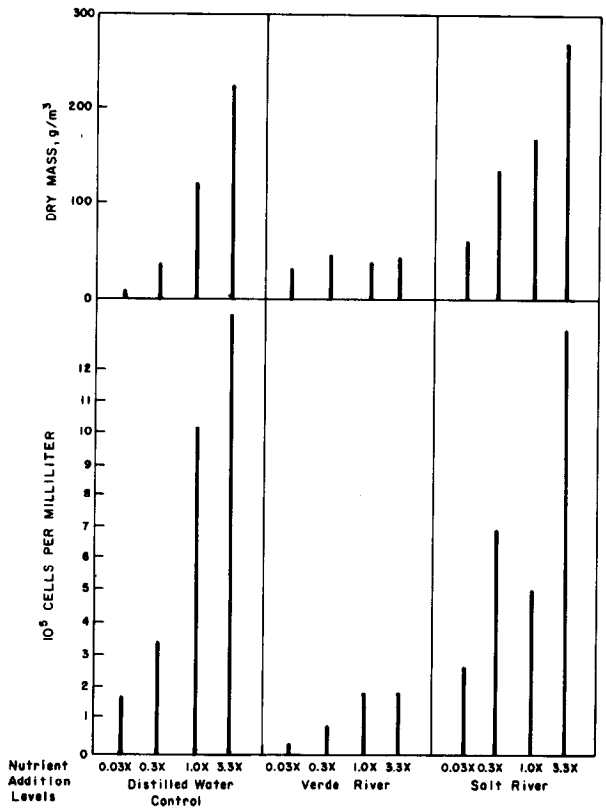


Figure 4.—21-day cell counts versus 21-day dry mass measurements. Mean values of three replications per nutrient level tested.

It has been observed that localized filamentous algae blooms seem to occur near a stationary weather monitoring raft anchored in the upper lake. This phenomenon occurs from mid to late summer and only on or near the raft. This growth is probably due to the warming of the epilimnion and to an abundance of gull droppings on the raft. Indications were that both lakes may be nutrient limited regarding algae production, and this question was investigated.

Water samples were collected from near the center of both lakes and processed in the normal manner. The phosphorus nutrient addition in one set of samples from each lake was deleted, and the nitrogen nutrient addition was deleted in another set of samples from each lake. The results of these nutrient limiting studies are shown in table 2 and on figure 5. This figure shows that both nitrogen and phosphorus appear to be limiting in this situation. Generally, the waters containing a nitrogen/phosphorus content ratio greater than 11.3:1 may be considered phosphorus limited, and waters containing N:P ratios of less than 11.3:1 can be considered nitrogen limited [11]. Other nutrients may be limiting at times, but these two elements are by far the most common. A high algal growth response from the addition of the various concentrations of the complete nutrient solution indicates that the Twin Lakes' water is indeed nutrient (P-N) limiting.

- Figure 6 illustrates a very good example of a eutrophic water collected from the South Platte River in Colorado that developed high

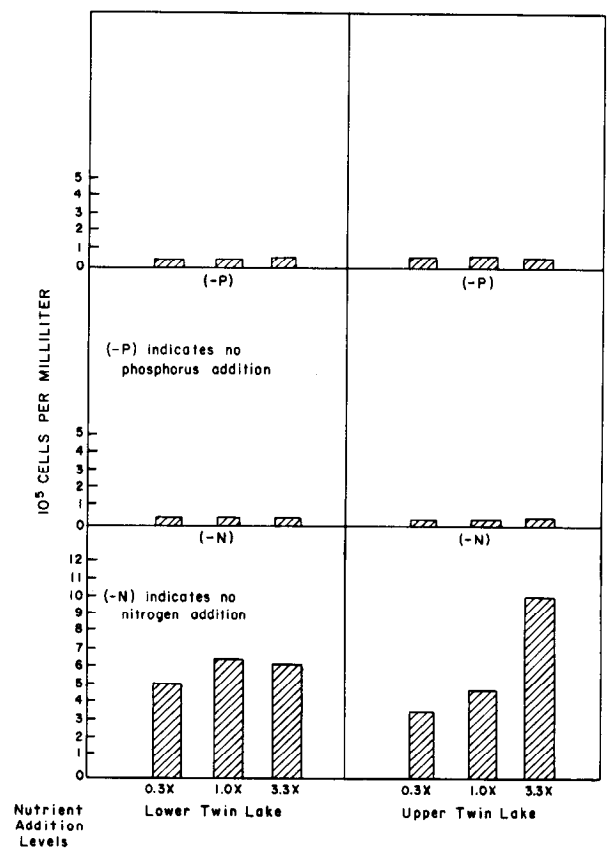


Figure 5.—21-day cell counts on nutrient limiting studies at Twin Lakes, Colo.

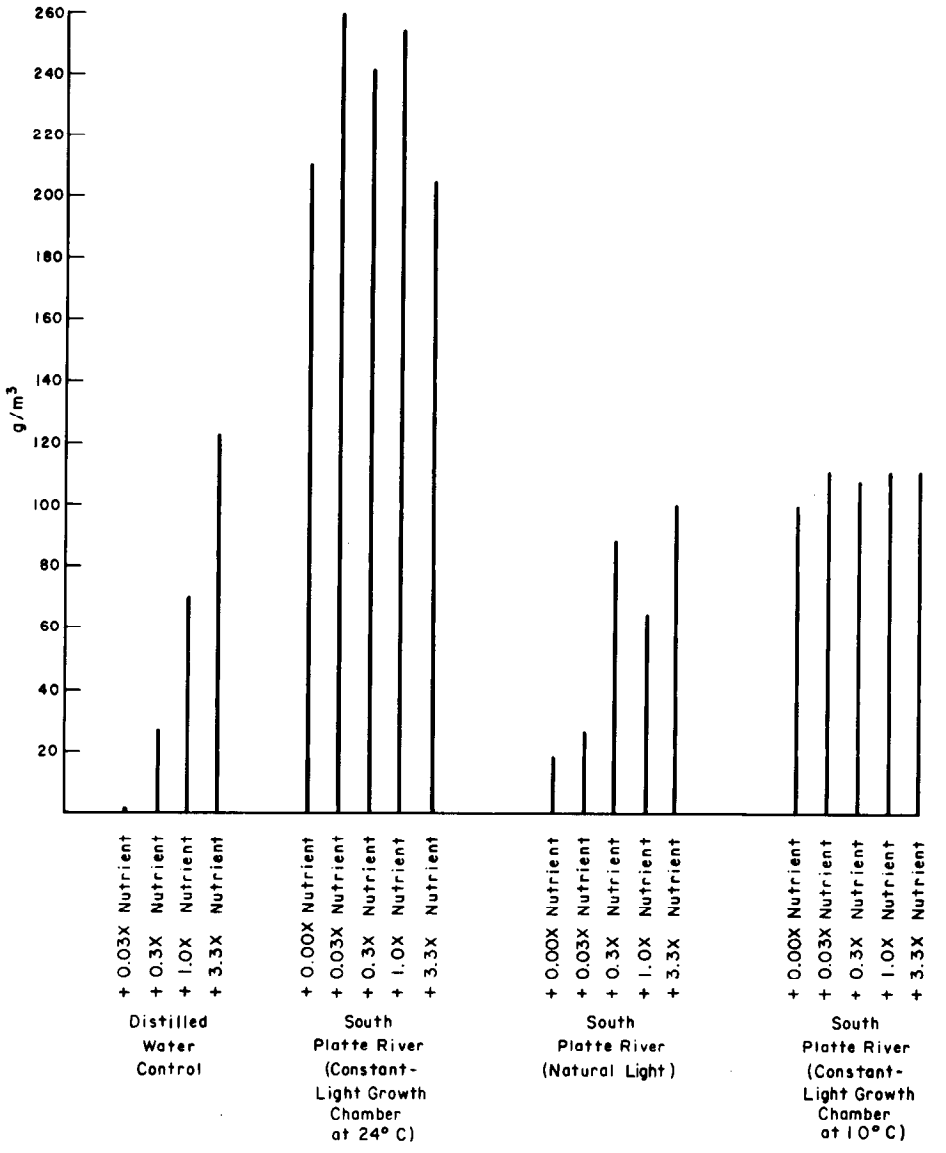


Figure 6.—21-day dry mass measurements. Mean values of three replications per nutrient level tested.

algal cell counts in raw water with no additions of nutrients. This is especially seen in the samples grown in a controlled light and temperature growth chamber. The South Platte River is the source water for the proposed Narrows Reservoir. Artificial substrate samplers for aquatic invertebrates and algae were also installed at the locations of the water samples. The SDI (standard diversity index) and the identification performed on the South Platte water supports the eutrophic classification found in the algal assay test (tables 3 and 4). Otto [10] stated that an SDI of less than 3.0 indicates a trend toward aquatic habitat enrichment.

- The four sampling locations in the Central Arizona Project (Salt, Verde, Colorado, and Gila Rivers) with different levels of salinity gave varying results. Orme Reservoir would store water from the Salt, Verde, and Colorado Rivers in varying amounts throughout the year and, as currently planned, the source water for the Buttes Reservoir will be the Gila River. The lower growth response as indicated by dry mass measurements and cell counts for the Verde and Colorado Rivers and, to some extent, the Gila River may relate to the higher sulfate and bicarbonate values (see tabs. 5 and 6 and fig. 7). These compounds may be binding with, and rendering unusable, the nutrient

Table 2.—*Results of Twin Lakes nutrient limiting AGP study of October 1976 showing mean values of replications*

| Sample tested[1] | 21-day dry mass, g/m³ | 7-day count, $10^3$ cells/mL | 7-day pH | 14-day count, $10^3$ cells/mL | 14-day pH | 21-day count $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|---|---|
| Distilled water + 0.03× nutrient | 0.0 | 44.0 | 7.45 | 41.1 | 7.30 | 48.1 | 7.68 |
| Distilled water + 0.3× nutrient | 31.8 | 46.2 | 7.83 | 131.0 | 8.15 | 252.7 | 7.55 |
| Distilled water + 1.0× nutrient | 107.8 | 114.8 | 10.45 | 258.2 | 10.45 | 456.2 | 8.05 |
| Distilled water + 3.3× nutrient | 99.2 | 166.4 | 10.55 | 318.3 | 9.93 | 582.0 | 8.23 |
| Station 2 + 0.3× nutrient | 70.0 | 208.1 | 10.28 | 468.7 | 8.50 | 504.3 | 8.10 |
| Station 2 + 1.0× nutrient | 153.9 | 193.3 | 10.13 | 554.2 | 10.05 | 654.8 | 8.33 |
| Station 2 + 3.3× nutrient | 104.0 | 199.4 | 10.55 | 498.3 | 8.73 | 563.2 | 8.45 |
| Station 4 + 0.3× nutrient | 54.0 | 199.6 | 10.45 | 305.3 | 8.50 | 370.2 | 8.15 |
| Station 4 + 1.0× nutrient | 176.4 | 173.8 | 10.50 | 482.3 | 10.20 | 483.0 | 8.30 |
| Station 4 + 3.3× nutrient | 164.0 | 231.3 | 10.59 | 616.4 | 10.43 | 1029.8 | 8.88 |
| Station 2 (−P)[2] + 0.3× nutrient | 2.1 | 44.7 | 8.18 | 54.5 | 8.10 | 32.5 | 8.08 |
| Station 2 (−P) + 1.0× nutrient | 4.2 | 47.0 | 8.20 | 44.2 | 8.28 | 37.9 | 8.05 |
| Station 2 (−P) + 3.3× nutrient | 3.9 | 49.6 | 8.43 | 47.5 | 8.50 | 42.2 | 8.33 |
| Station 4 (−P) + 0.3× nutrient | 6.8 | 53.4 | 8.25 | 50.3 | 8.20 | 51.5 | 8.03 |
| Station 4 (−P) + 1.0× nutrient | 2.9 | 51.5 | 8.25 | 43.0 | 8.38 | 41.5 | 8.22 |
| Station 4 (−P) + 3.3× nutrient | 0.6 | 49.6 | 8.43 | 41.9 | 8.50 | 44.0 | 8.28 |
| Station 2 (−N)[3] + 0.3× nutrient | 4.6 | 5.7 | 8.13 | 26.7 | 8.20 | 29.3 | 8.10 |
| Station 2 (−N) + 1.0× nutrient | 0.1 | 6.9 | 8.20 | 34.8 | 8.33 | 38.2 | 8.10 |
| Station 2 (−N) + 3.3× nutrient | 0.2 | 6.0 | 8.35 | 24.7 | 8.45 | 36.8 | 8.33 |
| Station 4 (−N) + 0.3× nutrient | 5.3 | 18.1 | 8.15 | 24.1 | 8.10 | 27.5 | 8.10 |
| Station 4 (−N) + 1.0× nutrient | 3.5 | 49.6 | 8.13 | 34.0 | 8.35 | 32.1 | 8.13 |
| Station 4 (−N) + 3.3× nutrient | 9.6 | 23.7 | 8.55 | 35.9 | 8.43 | 51.1 | 8.33 |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.
[2] (−P) Indicates no phosphorus addition
[3] (−N) Indicates no nitrogen addition

ions needed by the test alga. This would create a limiting condition for algal growth.

It was reported by Guseva [7, 12, 13] that concentrations of manganese in excess of 0.005 mg/L had a toxic effect on some algae. This seems highly unlikely, but the levels found in the Salt, Verde, and Gila Rivers may be somewhat inhibitory to the growth of the test algae. The levels found for copper, lead, iron, and zinc are below the documented toxic levels [14] and would not seem to be a problem.

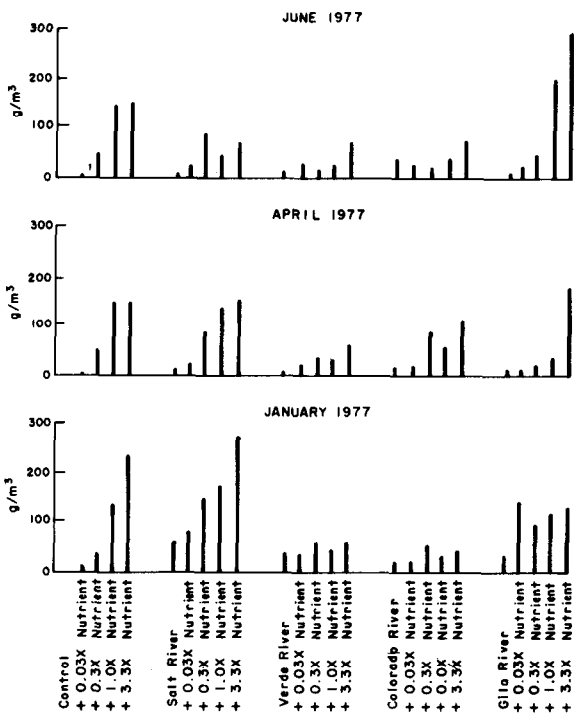


Figure 7.—21-day dry mass measurements on *Selenastrum capricornutum.*

None of the levels of the heavy metals found would appear to be toxic in themselves, but the levels indicated might be synergistic in various combinations and may produce an inhibitory effect on the growth of the test alga.

Algal growth is very sensitive to "available" nutrient levels, their ratios, and the levels of toxic materials present, and the exact cause or causes for subtle inhibition of growth is difficult to determine. Appendix E presents the results of an AABT study which does not appear to be consistent with field observations. It was previously concluded [15] that the algal assay results were not considered representative of actual water quality conditions at the time of sampling this particular water system. This may have been due to significant changes in the nutrient levels in the samples during shipment from the field to the laboratory.

Table 3.—*Standard diversity index of algae collected on glass slide samplers based on random field counts*

| Sampling Site | Calculated SDI |
|---|---|
| South Platte River at Masters | 1.0 |
| | 1.1 |
| | 1.4 |
| | 1.4 |
| | 1.6 |
| | 2.2 |
| South Platte River downstream of Fort Morgan diversion | 0.2 |
| | 0.4 |
| | 0.4 |
| | 0.5 |
| | 0.6 |
| | 0.7 |
| | 0.8 |
| | 0.8 |
| | 1.0 |
| | 1.2 |
| | 1.8 |

## DISCUSSION

In evaluating the AABT, it is evident that there are inherent limitations and bias within the test and that conclusive evidence must not be drawn on the basis of this single biological test. Instead, the AABT should be used in conjunction with as many other physical, chemical, and biological measurements as possible to accurately assess current situations as well as to predict possible future conditions [16].

The AABT is a valuable test procedure in that it provides a laboratory simulation showing trends in the primary production capacity of a natural water system and some of the effects of nutrient addition on that system.

Table 4.—*Identification of algae collected on glass slide samplers placed in South Platte River*

| Organism | South Platte River at Masters, percent of all taxa | South Platte River downstream of Fort Morgan diversion, percent of all taxa |
|---|---|---|
| *Navicula* sp. | 70.76 | 94.28 |
| *Synedra* sp. | 6.63 | 0.56 |
| *Cyclotella* sp. | 4.67 | 0.16 |
| *Diatoma* sp. | 0.24 | - |
| *Ulothrix* sp. | 0.98 | - |
| *Diatoma vulgare* | 5.90 | 0.36 |
| *Diplonia Smithii* | - | 3.84 |
| *Nitzschia* sp. | - | 0.01 |
| *Gyrosigma* sp. | 0.48 | 0.03 |
| *Gomphonema* sp. | 2.70 | 0.21 |
| *Fragilaria* sp. | 1.96 | 0.54 |
| Unidentified sp. | 5.68 | 0.01 |

It has been found that cell counting and dry mass measurements have been most representative of algal growth in our studies, and excellent correlations have been observed.

There has been considerable discussion concerning sterilization of test waters by autoclaving or filtration [17]. Autoclaving is a more rapid procedure, but it may change the water chemistry to some degree as shown in table 7. The major effects of autoclaving, as seen in the four samples in table 6, seem to be the rise in pH, possible precipitation of calcium, and a shift from a bicarbonate to a carbonate system. The increase in carbonate may cause some changes in algal growth response by the reduction of more readily available carbon.

Filtration of the test waters through a 0.45-micron filter will remove unwanted algae, but will also strip the waters of particulate matter as well as nutrients that might ultimately be released from any endemic species of algae and influence test algae growth response. It would seem that filtration would not be a desirable sterilization technique when high natural concentrations of algae are present.

Klotz, Cain, and Trainor [18] have shown that two principal problems exist with this laboratory algal assay. The first problem involves the test alga. The concentration of nutrients in the culture media is far higher than in most surface waters, and nutrient uptake in luxury amounts is a commonly known phenomenon with many species. This could provide an unrealistic and inaccurate measurement of algal growth in nutrient-poor waters because growth to some extent is supported intracellularly.

The second problem involves the size of the algal inoculum. If the inoculum is too small, the response in nutrient-poor waters will be insignificant and extremely difficult to measure. Conversely, if too large an inoculum is used, an erroneous high indication of algae growth will be measured.

There is a need for continued research in refining and standardizing the AABT when it is being used to predict impacts of water quality changes in reservoirs. It may well be that endemic species of algae would, in some instances, have to be used with a standardized nutrient solution, and natural environmental conditions may have to be more accurately simulated for improved accuracy in these predictions. It would be desirable to conduct further studies evaluating testing using other species of algae as the indicator organism. Until such time as a more precise technique can be developed, the present AABT will continue to be a worthwhile biological indicator of algal response to water quality, providing the limitations of the test are fully understood.

Table 5.—*Results of AGP tests in June 1977 showing mean values of three replications per test*

| Sample tested[1] | 21-day dry mass, g/m³ | 7-day count, $10^3$ cells/mL | 7-day pH | 14-day count, $10^3$ cells/mL | 14-day pH | 21-day count, $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|---|---|
| Distilled water + 0.03× nutrient | 0.0 | 14.6 | 6.30 | 43.8 | 6.64 | 50.2 | 6.43 |
| Distilled water + 0.3× nutrient | 47.7 | 91.8 | 8.70 | 404.1 | 7.31 | 435.3 | 7.76 |
| Distilled water + 1.0× nutrient | 130.3 | 196.4 | 10.38 | 870.0 | 10.39 | 993.3 | 10.41 |
| Distilled water + 3.3× nutrient | 136.0 | 297.7 | 10.52 | 829.2 | 10.59 | 999.9 | 8.36 |
| Salt River | 2.8 | 4.8 | 8.20 | 13.0 | 8.58 | 10.9 | 8.53 |
| Salt River + 0.03× nutrient | 10.8 | 16.7 | 8.40 | 27.0 | 8.58 | 29.4 | 8.57 |
| Salt River + 0.3× nutrient | 45.8 | 94.2 | 8.45 | 166.3 | 8.45 | 203.3 | 8.60 |
| Salt River + 1.0× nutrient | 33.7 | 118.1 | 8.58 | 212.7 | 8.47 | 179.1 | 8.48 |
| Salt River + 3.3× nutrient | 64.6 | 93.3 | 9.49 | 192.0 | 8.47 | 265.8 | 8.48 |
| Verde River | 3.6 | 11.6 | 8.81 | 10.4 | 8.71 | 10.4 | 8.67 |
| Verde River + 0.03× nutrient | 13.7 | 8.1 | 8.62 | 9.7 | 8.66 | 13.3 | 8.61 |
| Verde River + 0.3× nutrient | 7.0 | 8.2 | 8.57 | 7.7 | 8.70 | 18.9 | 8.68 |
| Verde River + 1.0× nutrient | 13.1 | 48.8 | 8.70 | 49.8 | 8.76 | 47.8 | 8.75 |
| Verde River + 3.3× nutrient | 64.9 | 111.0 | 9.90 | 161.5 | 9.15 | 190.0 | 8.87 |
| Colorado River | 21.1 | 2.1 | 8.62 | 3.9 | 8.50 | 6.8 | 8.50 |
| Colorado River + 0.03× nutrient | 14.8 | 21.0 | 8.45 | 46.8 | 8.45 | 46.4 | 8.53 |
| Colorado River + 0.3× nutrient | 8.3 | 44.6 | 8.44 | 18.5 | 8.40 | 26.7 | 8.51 |
| Colorado River + 1.0× nutrient | 23.6 | 34.4 | 8.76 | 103.3 | 8.54 | 67.1 | 8.59 |
| Colorado River + 3.3× nutrient | 49.2 | 102.9 | 8.97 | 362.0 | 8.74 | 225.0 | 8.58 |
| Gila River | 3.4 | 25.8 | 8.50 | 40.9 | 8.42 | 51.7 | 8.45 |
| Gila River + 0.03× nutrient | 11.0 | 46.8 | 8.35 | 75.9 | 7.05 | 100.9 | 8.40 |
| Gila River + 0.3× nutrient | 39.4 | 215.6 | 8.58 | 265.2 | 7.02 | 246.7 | 8.54 |
| Gila River + 1.0× nutrient | 176.9 | 384.8 | 10.20 | 464.6 | 9.89 | 826.2 | 9.63 |
| Gila River | 269.5 | 446.3 | 10.56 | 572.3 | 9.36 | 888.0 | 9.04 |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.

Table 6.—*Chemical analyses of field waters in June 1977*

| Parameter[1] | Salt River, mg/L | Verde River, mg/L | Colorado River, mg/L | Gila River, mg/L |
|---|---|---|---|---|
| TDS | 702.00 | 336.00 | 758.00 | 2720.00 |
| Calcium | 53.20 | 43.00 | 81.40 | 162.00 |
| Magnesium | 14.40 | 26.80 | 27.40 | 67.00 |
| Sodium | 188.00 | 29.40 | 113.00 | 689.00 |
| Potassium | 3.52 | 1.95 | 3.52 | 9.78 |
| Carbonate | 0.00 | 0.00 | 0.00 | 0.00 |
| Bicarbonate | 149.00 | 242.00 | 160.00 | 239.00 |
| Sulfate | 51.40 | 42.70 | 282.00 | 472.00 |
| Chloride | 301.00 | 20.60 | 98.00 | 1000.00 |
| Anions and cations | 761.00 | 406.00 | 765.00 | 2640.00 |
| Copper | 0.003 | <0.002 | <0.002 | 0.010 |
| Lead | <0.002 | <0.002 | 0.003 | 0.020 |
| Iron | 0.010 | 0.015 | 0.005 | 0.960 |
| Manganese | 0.010 | 0.030 | <0.005 | 0.390 |
| Zinc | 0.003 | 0.002 | 0.005 | 0.005 |
| Conductivity, μS/cm | 1310.00 | 533.00 | 1110.00 | 4600.00 |
| pH | 7.90 | 8.30 | 8.20 | 7.70 |

[1] Analyses by "Standard Methods for Examination of Water and Wastewater, 14th Edition," APHA, AWWA, WPCF, 1975.

Table 7.—*Raw river waters versus autoclaved river waters. Chemical composition in mg/L*

| Sample | pH | TDS | Ca | Mg | Na | K | $CO_3$ | $HCO_3$ | $SO_4$ | Cl | Anions and cations | Cu | Pb | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Verde River | 8.30 | 336 | 43.0 | 26.8 | 29.4 | 1.95 | 0.00 | 242 | 42.7 | 20.6 | 406 | <0.002 | <0.002 | 0.015 | 0.030 | 0.002 |
| Autoclaved | 8.50 | 262 | 14.0 | 26.7 | 29.4 | 1.95 | 7.50 | 146 | 48.0 | 21.3 | 295 | 0.007 | 0.002 | 0.015 | 0.030 | 0.002 |
| Salt River | 7.90 | 702 | 53.2 | 14.4 | 188.0 | 3.52 | 0.00 | 149 | 51.4 | 301.0 | 761 | 0.003 | <0.002 | 0.010 | 0.010 | 0.003 |
| Autoclaved | 8.30 | 708 | 38.4 | 15.4 | 194.0 | 3.91 | 0.00 | 107 | 44.6 | 301.0 | 705 | <0.002 | <0.002 | 0.010 | 0.020 | 0.003 |
| Gila River | 7.70 | 2720 | 162.0 | 67.0 | 689.0 | 9.78 | 0.00 | 239 | 472.0 | 1000.0 | 2640 | 0.010 | 0.020 | 0.960 | 0.390 | 0.005 |
| Autoclaved | 8.30 | 2770 | 153.0 | 59.2 | 729.0 | 10.20 | 0.00 | 132 | 450.0 | 1110.0 | 2640 | 0.008 | 0.030 | 0.920 | 0.390 | 0.005 |
| Colorado River | 8.20 | 758 | 81.4 | 27.4 | 113.0 | 3.52 | 0.00 | 160 | 282.0 | 98.1 | 765 | <0.002 | 0.003 | 0.005 | <0.005 | 0.005 |
| Autoclaved | 8.50 | 692 | 64.2 | 29.6 | 117.0 | 3.52 | 3.30 | 100 | 284.0 | 89.5 | 691 | 0.003 | 0.003 | 0.010 | <0.005 | 0.005 |

## BIBLIOGRAPHY


[1] National Academy of Sciences. *Eutrophication: Causes, Consequences, and Correctives,* Washington, D.C., p. 661, 1969.

[2] MacKenthun, K. M. ed., *The Practice of Water Pollution Biology,* U.S. Department of the Interior, FWPCA, Washington, D.C., pp. 221-225, 1969.

[3] *Standard Methods for the Examination of Water and Wastewater,* American Public Health Association, Inc., New York, pp. 744-758, 1975.

[4] "Interlaboratory Precision Test - An Eight-Laboratory Evaluation of the Provisional Algal Assay Procedure Bottle Test," U.S. Environmental Protection Agency, National Eutrophication Research Program, Corvallis, Oreg., p. 70, 1971.

[5] "The *Selenastrum capricornutum* Printz Algal Assay Bottle Test," U.S. Environmental Protection Agency, Environmental Research Laboratory Report, EPA-600/9-78-018, Corvallis, Oreg., p. 126, 1978.

[6] Stein, J. R., ed., *Handbook of Phycological Methods, Culture Methods, and Growth Measurements,* Cambridge University Press, London, pp. 327-329, 1973.

[7] Shoaf, W. T., and B. W. Lium, "An Evaluation of Methods for Measuring Algal Growth," *Journal of Research of U.S. Geological Survey,* vol. 4, No. 4, pp. 497-504, July-August 1976.

[8] Stumm, W., and J. J. Morgan, *Aquatic Chemistry,* Wiley-Interscience, A Division of John Wiley and Sons, Inc., New York, pp. 118-160, 1970.

[9] Hannan, P. J., *A Reliable Algal Assay Procedure Based on pH Measurements,* Proc. of the Conference on Marine Biology in Environmental Protection held at San Clemente Island, Calif., pp. 71-78, 1973.

[10] Otto, N. E., *Investigations of Aquatic Weed Control Methods and Their Environmental Effects on Nontarget Aquatic Species,* Bureau of Reclamation Report REC-ERC-74-11, Denver, Colo., p. 50, 1974.

[11] Miller, W. E., J. C. Greene, and J. Shiroyama, "Application of Algal Assays to Define the Effects of Wastewater Effluents Upon Algal Growth in Multiple Use River Systems," In: *Biostimulation and Nutrient Assessment,* edited by Middlebrooks, E. J., D. H. Falkenborg, and T. E. Maloney, Ann Arbor Science, Ann Arbor, Mich., pp. 77-92, 1975.

[12] Guseva, K. A. "Hydrobiology and Microbiology of the Uchinskii Reservoir (Moscow-Volga Canal), II, Observations of the Department of *Anabaena Lemmermannii, Aphanizomenen flos aquae,* and *Asterionella formosa.*" Mikrobiologiya 6,449 (1937); Water Pollution Abs. 20 February 1947.

[13] Guseva, K. A., "Water-Bloom in the Uchinskii Reservoir." Bull. Soc. Nat. (Moscow) 48,4:30 (1939; Water Pollution Abs.) November 1940.

[14] McKee, J. E., and H. W. Wolf, *Water Quality Criteria.* Second Edition. Publ. No. 3-A California State Water Resources Control Board, Sacramento, 548 pages, reprinted June 1, 1976.

[15] "Report on Boysen Reservoir, Fremont County, Wyoming, EPA Region VIII Working Paper No. 883," U.S. Environmental Protection Agency National Eutrophication Survey, Corvallis, Oreg., p. 47, July, 1977.

[16] Thornton, K. W. "Approaches for Assessing Eutrophication Potential in Reservoirs," U.S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss., Miss. Misc. Paper 0-77-3, 1977.

[17] Shiroyama, T., W. E. Miller, and J. C. Greene, "Comparison of the Algal Growth Response in *Selenastrum capricornutum* Printz and *Anabgena flos-aquae* (Lyngb.) DeBrebisson in Waters

Collected From Shagawa Lake, Minnesota," In: *Biostimulation and Nutrient Assessment*, edited by Middlebrooks, E. J., D. H. Falkenborg, and T. E. Maloney, Ann Arbor Science, Ann Arbor, Mich., pp. 127-148, 1975.

[18] Klotz, R. L., J. R. Cain, and F. R. Trainor, "A Sensitive Algal Assay: An Improved Method for Analysis of Freshwaters," *Journal of Phycology,* vol. 11, pp. 411-414, 1975.

# APPENDIXES

# RESULTS OF AABT STUDIES COMPLETED ON FOUR FIELD WATER SOURCES

# APPENDIX A

# BIG SIOUX RIVER — SLIP-UP CREEK AABT RESULTS (FIRST STUDY)

## INTRODUCTION

This report presents progress on an AGP (algae growth potential) evaluation of water collected from the Big Sioux River and Slip-Up Creek near Sioux Falls, S. Dak.

The water samples were collected on April 16, 1976 and represent the early spring runoff that will eventually be diverted to a proposed potable water reservoir on Slip-Up Creek.

## RESULTS AND DISCUSSION

The results of the laboratory AGP studies given in table A-1 show that the Big Sioux River and Slip-Up Creek support growth of the test algae corresponding to somewhat less than the 0.3X standard nutrient solution levels of production. Spiking of the river water with nutrient solution from the 0.03X to the 3.3X level shows a tremendous increase in algae production with increased nutrient availability. The algae growth potential with the addition of various standard nutrient spikes was much greater for Slip-Up Creek than for the Big Sioux River.

Submitted water samples were analyzed for chemical composition, and the results of these analyses are given in table A-2.

The water from Slip-Up Creek was less turbid than that from the Big Sioux River. Eight species of diatoms, but in limited numbers, were revealed by microscopic examination. The Big Sioux River also contained eight species of diatoms along with some filamentous and unicellular green algae evident.

The data from the algae assay test suggests that the field waters are relatively productive. Further enrichment with additional nutrients will result in significant increases in algae growth that is nutrient concentration dependent.

Table A-1.—*Results of AGP tests—Big Sioux River—Slip-Up Creek (First study). Mean values of replications*

| Sample tested | 21-day dry mass, g/m³ | 21-day chlorophyll, mg/m³ | 8-day count, $10^3$ cells/mL | 8-day pH | 21-day count, $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|---|
| Distilled water[1] | 22.6 | N.D.[2] | 5.6 | 8.65 | 22.3 | 9.44 |
| + 0.3× nutrient | | | | | | |
| Distilled water | 46.8 | 29.56 | 5.5 | 8.68 | 145.9 | 9.64 |
| + 1.0× nutrient | | | | | | |
| Distilled water | 160.2 | 116.60 | 46.4 | 9.96 | 342.4 | 10.36 |
| + 3.3× nutrient | | | | | | |
| Big Sioux River | 15.2 | N.D. | 7.6 | 8.61 | 9.1 | 8.46 |
| River water | 29.6 | N.D. | 7.5 | 8.62 | 12.4 | 8.46 |
| + 0.03× nutrient | | | | | | |
| River water | 101.9 | N.D. | 12.2 | 9.90 | 47.4 | 8.66 |
| + 0.3× nutrient | | | | | | |
| River water | 193.6 | 20.36 | 47.3 | 10.08 | 143.2 | 9.18 |
| + 1.0× nutrient | | | | | | |
| River water | 275.7 | 48.95 | 118.85 | 9.95 | 290.2 | 9.32 |
| + 3.3× nutrient | | | | | | |
| Slip-Up Creek | 5.3 | N.D. | 4.5 | 8.56 | 8.8 | 8.66 |
| Creek water | 13.1 | N.D. | 8.2 | 8.95 | 33.2 | 8.72 |
| + 0.03× nutrient | | | | | | |
| Creek water | 80.1 | 8.82 | 96.3 | 10.09 | 171.7 | 9.00 |
| + 0.3× nutrient | | | | | | |
| Creek water | 158.4 | 58.83 | 121.3 | 10.36 | 275.0 | 9.29 |
| + 1.0× nutrient | | | | | | |
| Creek water | 282.3 | 85.83 | 148.3 | 10.19 | 348.9 | 9.29 |
| + 3.3× nutrient | | | | | | |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.
[2] N.D. denotes no data. May have resulted from the senecence of algae and the subsequent loss or reduction of chlorophyll.

Table A-2.—*Chemical analyses of field waters —Big Sioux River—Slip-Up Creek (First study)*

| Parameter | Big Sioux River, mg/L | Slip-Up Creek, mg/L |
|---|---|---|
| TDS[1] | 556.00 | 426.00 |
| Calcium[1] | 88.40 | 65.60 |
| Magnesium[1] | 36.80 | 32.30 |
| Sodium[1] | 22.80 | 14.70 |
| Potassium[1] | 6.26 | 5.08 |
| Carbonate[1] | — | — |
| Bicarbonate[1] | 216.00 | 293.00 |
| Sulfate[1] | 194.00 | 73.90 |
| Chloride[1] | 25.60 | 11.40 |
| Anions + cations[1] | 589.69 | 495.81 |
| Nitrate[2] | 1.30 | 0.60 |
| Nitrite[2] | 0.01 | 0.01 |
| Orthophosphate[2] | 0.08 | 0.06 |
| Conductivity, $\mu$S/cm | 775.00 | 662.00 |
| pH | 7.70 | 7.90 |

[1] Analyses by "Standard Methods for Examination of Water and Wastewater," - APHA, 1965.
[2] Analyses by "Water Analysis Procedures," Direct Reading, Hach Chemical Company.

# APPENDIX B

# BIG SIOUX RIVER — SLIP-UP CREEK AABT RESULTS (SECOND STUDY)

## INTRODUCTION

This is the second report on an AGP evaluation of water collected form the Big Sioux River and Slip-Up Creek near Sioux Falls, S. Dak.

The water samples were collected on June 2, 1976 and represent the normal summer flow that will eventually be diverted to a proposed potable water reservoir on Slip-Up Creek.

## RESULTS AND DISCUSSION

The results of the laboratory AGP studies given in table B-1 show that the Big Sioux River and Slip-Up Creek support growth of the test alga corresponding to somewhat less than the 0.3X standard nutrient solution levels of production. Spiking of the river water with nutrient solution from the 0.03X to the 3.3X level shows a tremendous increase in algae production with increased nutrient availability. This is particularly apparent for Slip-Up Creek.

Microscopic examination revealed seven species of diatoms in water from the Big Sioux River and four species of diatoms from the Slip-Up Creek water sample. *Cyclotella meneghiniana* was the dominant species in both samples.

Submitted water samples were analyzed for nitrogen and phosphorus content. Results of these analyses are given in table B-2.

The data from the algal assay test suggest that the field waters are relatively productive. Further enrichment with additional nutrients will result in significant increases in algal growth that is nutrient concentration dependent.

Table B-1.—*Results of AGP tests—Big Sioux River—Slip-Up Creek (Second study). Mean values of replications*

| Sample tested[1] | 21-day dry mass, g/m³ | 21-day chlorophyll, mg/m³ | 8-day count, $10^3$ cells/mL | 8-day pH | 21-day count, $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|---|
| Distilled water | 0.0 | 0.00 | 0.95 | 6.22 | 1.25 | 6.18 |
| Distilled water + 0.3× nutrient | 27.0 | 5.63 | 24.70 | 8.10 | 25.40 | 7.03 |
| Distilled water + 1.0× nutrient | 60.1 | 51.40 | 1.95 | 7.70 | 34.55 | 9.00 |
| Distilled water + 3.3× nutrient | 233.0 | [2]11.88 | 84.40 | 10.84 | 411.35 | 7.70 |
| Big Sioux River | 33.7 | 0.00 | 6.55 | 8.62 | 10.10 | 8.43 |
| River water + 0.03× nutrient | 41.2 | 0.00 | 8.05 | 8.63 | 14.95 | 8.55 |
| River water + 0.3× nutrient | 16.6 | 4.19 | 6.20 | 8.64 | 17.25 | 8.63 |
| River water + 1.0× nutrient | 263.5 | 20.83 | 197.80 | 10.73 | 277.40 | 8.59 |
| River water + 3.3× nutrient | 384.4 | 124.30 | 107.10 | 9.97 | 453.55 | 10.16 |
| Slip-Up Creek | 10.3 | 0.00 | 4.90 | 8.59 | 7.80 | 8.63 |
| Creek water + 0.03× nutrient | 81.0 | 0.00 | 73.70 | 9.08 | 112.85 | 8.69 |
| Creek water + 0.3× nutrient | 143.7 | 0.00 | 121.80 | 9.51 | 238.70 | 8.70 |
| Creek water + 1.0× nutrient | 282.7 | 22.30 | 259.10 | 10.80 | 536.30 | 8.86 |
| Creek water + 3.3× nutrient | 523.4 | 349.69 | 166.90 | 10.85 | 662.80 | 10.96 |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.

[2] Low value may have resulted from the senescence of algae and the subsequent reduction of chlorophyll. Pheophytin analysis may be performed in future algal assay tests to determine the presence of this degradation product.

Table B-2.—*Chemical analyses of field waters —Big Sioux River—Slip-Up Creek (Second study)*

| Parameter[1] | Big Sioux River | | Slip-Up Creek | |
|---|---|---|---|---|
| pH | 8.10 | | 8.05 | |
| Nitrate, | 0.30 | mg/L | 0.40 | mg/L |
| Nitrite, | 0.015 | mg/L | 0.025 | mg/L |
| Ortho-phosphate | 0.07 | mg/L | 0.30 | mg/L |

[1] Analyses by "Water Analysis Procedures," Direct Reading, Hach Chemical Company

# APPENDIX C

## UNCOMPAHGRE RIVER AABT RESULTS (FIRST AND SECOND STUDIES)

## INTRODUCTION

This report combines two AGP studies of water collected from the Uncompahgre River near Grand Junction, Colo.

The water samples were collected on June 2 and 22, 1976, and represent the source water for the proposed Ridgway Reservoir on the Uncompahgre River.

## RESULTS AND DISCUSSION

The results of the laboratory AGP studies given in tables C-1 and C-2 show that the Uncompahgre River supports growth of the test alga corresponding to the 0.3X standard nutrient solution level of production. Spiking of the river water with nutrient solution from the 0.03X to the 3.3X level shows a tremendous increase in algae production with increased nutrient availability. The two water samples supported similar algal growth responses in these algal assay tests.

Microscopic examination revealed seven species of diatoms in the first water sample and six species in the second sample.

The water sample of June 2, 1976, was analyzed for chemical composition and for the presence of heavy metals. The results of these analyses are given in table C-3.

The water sample submitted on June 22, 1976, was only analyzed for nitrogen and phosphorus content. Results of these analyses are given in table C-4.

The data from these algal assay tests suggest that the field waters are relatively productive. Further enrichment with additional nutrients will result in significant increases in algal growth that is nutrient concentration dependent. The levels of heavy metals found in the June 2 sample did not appear to inhibit algal growth.

Table C-1.—*Results of AGP tests—Uncompahgre River (First study). Mean values of replications*

| Sample tested[1] | 21-day dry mass, g/m³ | 21-day chlorophyll, mg/m³ | 8-day count, $10^3$ cells/mL | 8-day pH | 21-day count, $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|---|
| Distilled water + 0.3× nutrient | 27.0 | 5.63 | 24.7 | 8.10 | 25.4 | 7.03 |
| Distilled water + 1.0× nutrient | 60.1 | 51.40 | 1.9 | 7.70 | 34.5 | 9.00 |
| Distilled water + 3.3× nutrient | 233.0 | [2] 11.88 | 84.4 | 10.84 | 411.3 | 7.70 |
| Uncompahgre River | 38.0 | 0.00 | 84.4 | 10.84 | 411.3 | 7.70 |
| River water + 0.03× nutrient | 77.2 | 7.67 | 14.0 | 8.62 | 48.4 | 8.32 |
| River water + 0.3× nutrient | 180.3 | 0.00 | 100.8 | 9.53 | 98.5 | 8.09 |
| River water + 1.0× nutrient | 326.6 | 9.60 | 86.6 | 10.81 | 194.2 | 8.41 |
| River water + 3.3× nutrient | 378.3 | 421.95 | 167.0 | 10.97 | 312.8 | 10.25 |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.

[2] Low value may have resulted from the senescence of algae and the subsequent reduction of chlorophyll. Pheophytin analysis may be performed in future algal assay tests to determine the presence of this degradation product.

Table C-2.—*Results of AGP tests—Uncompahgre River (Second study). Mean values of replications*

| Sample tested[1] | 21-day dry mass, g/m³ | 21-day chlorophyll, mg/m³ | 8-day count, $10^3$ cells/mL | 8-day pH | 21-day count, $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|---|
| Distilled water + 0.3× nutrient | 46.4 | 2.50 | 6.0 | 7.72 | 16.7 | 7.92 |
| Distilled water + 1.0× nutrient | 111.7 | 0.47 | 26.0 | 10.57 | 135.6 | 8.00 |
| Distilled water + 3.3× nutrient | 231.0 | 47.31 | 50.6 | 10.98 | 321.4 | 8.74 |
| Uncompahgre River | 29.3 | 0.00 | 7.0 | 8.23 | 9.3 | 8.40 |
| River water + 0.03× nutrient | 61.4 | 0.00 | 27.6 | 8.40 | 38.3 | 8.46 |
| River water + 0.3× nutrient | 181.2 | 0.00 | 42.5 | 9.84 | 63.7 | 8.27 |
| River water + 1.0× nutrient | 262.3 | 17.52 | 82.5 | 10.78 | 170.2 | 8.39 |
| River water + 3.3× nutrient | 382.4 | 65.60 | 159.1 | 10.97 | 612.2 | 9.02 |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.

Table C-3.—*Chemical analyses of field waters —Uncompahgre River (First study)*

| Parameter | Uncompahgre River, mg/L |
|---|---|
| TDS[1] | 244.00 |
| Calcium[1] | 51.40 |
| Magnesium[1] | 3.42 |
| Sodium[1] | 8.97 |
| Potassium[1] | 1.96 |
| Carbonate[1] | 0.00 |
| Bicarbonate[1] | 64.10 |
| Sulfate[1] | 110.00 |
| Chloride[1] | 1.42 |
| Anions + cations[1] | 241.75 |
| Lead[1] | <0.10 |
| Copper[1] | 0.04 |
| Zinc[1] | 0.14 |
| Silver[1] | <0.005 |
| Nitrate[2] | 0.30 |
| Nitrite[2] | 0.005 |
| Orthophosphate[2] | 0.10 |
| Conductivity, μS/cm | 336.00 |
| pH | 7.80 |

[1] Analyses by "Standard Methods for Examination of Water and Wastewater," - APHA, 1965.
[2] Analyses by "Water Analysis Procedures," Direct Reading, Hach Chemical Company

Table C-4.—*Chemical analyses of field waters —Uncompahgre River (Second study)*

| Parameter[1] | Uncompahgre River |
|---|---|
| pH | 8.20 |
| Nitrate | 0.30 mg/L |
| Nitrite | 0.008mg/L |
| Orthophosphate | 0.12 mg/L |

[1] Analyses by "Water Analysis Procedures," Direct Reading, Hach Chemical Company

# APPENDIX D

# UNCOMPAHGRE RIVER AABT RESULTS (THIRD STUDY)

## INTRODUCTION

This report presents the results of the third AGP study conducted on a water sample collected on August 24, 1976, from the Uncompahgre River near Grand Junction, Colo., and summarizes the results of the three separate AGP tests conducted on water samples collected from this water source from June through August 1976.

The water samples used in these tests represent the source water for the proposed Ridgway Reservoir.

## RESULTS

### AGP Test Results of August 24, 1976 Sample

The results of the third laboratory AGP study given in table D-1 show that the Uncompahgre River supports growth of the test alga corresponding to the 0.3X comparative standard nutrient solution level of algae growth. Spiking of the river water with nutrient solution from the 0.03X to the 3.3X level shows an extensive increase in algal production with an increased nutrient availability.

The water sample was analyzed for nitrogen and phosphorus content, and the results of these analyses are given in table D-2.

The data from this AGP test suggest that the river water will support phytoplankton growth and is relatively productive. Further enrichment with additional nutrients will result in significant increases in algal growth that is nutrient concentration dependent.

### Results of Seasonal AGP Tests

In summarizing the results of the three AGP studies completed on water samples collected on June 2, June 22, and August 24, 1976, there appeared to be only minor differences in algal growth response in the three separate tests. In all cases, the AGP of the Uncompahgre River was similar to the productivity of the 0.3X comparative standard nutrient level. Biomass and cell count at the end of the 21-day culture period appear to be the best indicators of algal growth.

The averaging of the three individual studies, as given in table D-3, indicate statistically significant differences between the various nutrient spiked samples of water in both the distilled water checks and the Uncompahgre River waters. The 5 percent level is coded alphabetically to show confidence intervals of the sample replications distributed around the given mean values. The Uncompahgre River water alone shows excellent similarity to the 0.3X nutrient level control. Increases in nutrient additions to the river water supported a near linear increase in algal cell counts. Biomass shows a curvilinear response in algae production when nutrient levels were increased.

## DISCUSSION

There did not appear to be any inhibitory or toxic effect on the growth of *Selenastrum capricornutum* under conditions of these tests that might be attributed to the presence of ionized heavy metals occurring in the field waters.

Our chemical analysis of the June 2 water sample showed the water contained $<0.10$ mg/L of lead and $<0.005$ mg/L of silver. These levels are less than the detectable limits.

Zinc was found to be present at a concentration of 0.14 mg/L in this sample. It has been reported [1][1] that concentrations of 0.1 to 1.0 mg/L can be lethal to certain aquatic organisms in soft water, but calcium is antagonistic toward such toxicity. In this sample, calcium occurred at a concentration of 51.4 mg/L, a level that would likely make zinc nontoxic.

The toxicity of copper to aquatic organisms varies significantly with species and, like many heavy metals, it is strongly influenced by the physical and chemical characteristics of the water [2, 3]. The June 2 water sample was found to contain 0.04 mg/L of copper. This level of total copper is lower than many surface waters of the Western United States, as Braidech [4] reports values of 0.1 to 0.6 mg/L.

[1] Numbers in brackets refer to entries in the bibliography.

Concentrations of 0.015 to 3.0 mg/L have been reported as toxic to phytoplankton, particularly in soft water [1]. This toxic effect is greatly reduced in high alkaline water by the chemical combination of copper with the bicarbonate anion resulting in precipitation as copper carbonate [3, 5].

The 1971 water quality data submitted by project personnel reveal that iron did not exceed 0.68 mg/L in any of the water samples analyzed. Ellis [6] reported that 95 percent of the waters that support good fish fauna in the United States have a concentration of 0.7 mg/L of iron.

The results of these laboratory tests to determine algal growth potential of the Uncompahgre River water samples do not suggest the presence of heavy metals in a toxic form that would inhibit algal growth. It must always be taken into account that manipulation of the field water through collection, shipping, autoclaving, and finally to exposure of test algae might possibly alter overall water chemistry causing a heavy metal to become combined with other ions if it was originally in a free ionic form. However, we have no evidence of this happening in any of our laboratory AGP studies, particularly in dealing with alkaline field waters.

## BIBLIOGRAPHY


[1] McKee, J. E., and H. W. Wolf, *Water Quality Criteria,* Second Edition. Publ. No. 3-A California State Water Resources Control Board, Sacramento, 548 pages, 1963.

[2] Tarzwell, C. M., "Water Quality Criteria for Aquatic Life." *Biological Problems in Water Pollution,* RATSEC, Public Health Service, 246 (1957).

[3] Trama, F. B., "The Acute Toxicity of Copper to the Common Bluegill *(Lepomis Macrochirus Rafinesque).*" Notulae Naturae Academy of Natural Sciences of Philadelphia, Paper No. 257, Feb. 17, 1954.

[4] Braidech, M. W., "The Spectrographic Determination of Minor Chemical Constituents in Various Water Supplies in the United States," AWWA vol. 27, No. 5, 1935.

[5] Hale, F. E., "Relation of Copper and Brass Pipe to Health," *Water Works Eng.* 95, 240, 84, 139, 187 (1942).

[6] Ellis, M. W., "Water Conditions Affecting Aquatic Life in Elephant Butte Reservoir," U.S. Department of the Interior Bull. No. 34 (1940).


Table D-1.—*Results of AGP tests—Uncompahgre River (Third study). Mean values of replications*

| Sample tested[1] | 21-day dry mass, g/m³ | 21-day chlorophyll, mg/m³ | 13-day count, $10^3$ cells/mL | 21-day count, $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|
| Distilled water + 0.3× nutrient | 28.3 | 1.16 | 5.3 | 19.8 | 7.03 |
| Distilled water + 1.0× nutrient | 106.7 | 5.80 | 53.2 | 72.7 | 7.52 |
| Distilled water + 3.3× nutrient | 302.6 | 33.64 | 423.1 | 318.8 | 7.72 |
| Uncompahgre River | 29.7 | 0.00 | 8.4 | 16.7 | 7.81 |
| River water + 0.03× nutrient | 16.8 | 0.00 | 10.7 | 16.4 | 8.00 |
| River water + 0.3× nutrient | 44.1 | 0.00 | 38.4 | 67.5 | 8.06 |
| River water + 1.0× nutrient | 314.5 | 11.60 | 155.7 | 170.2 | 8.22 |
| River water + 3.3× nutrient | 369.4 | 95.12 | 243.2 | 434.3 | 8.17 |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.

Table D-2.—*Chemical analyses of field waters —Uncompahgre River (Third study)*

| Parameter[1] | Uncompahgre River |
|---|---|
| pH | 7.90 |
| Nitrate | 0.30 mg/L |
| Nitrite | 0.01 mg/L |
| Orthophosphate | 0.02 mg/L |

[1] Analyses by "Water Analysis Procedures," Direct Reading, Hach Chemical Company

Table D-3.—*Summary results of seasonal AGP tests. Average of six replications per parameter and nutrient level*

| | Cell count, $10^3$ cells/mL | Dry mass, g/$m^3$ |
|---|---|---|
| Distilled water + 0.3× nutrient | 21.5 a[1] | 33.90 ab[2] |
| Distilled water + 1.0× nutrient | 80.6 c | 92.82 d |
| Distilled water + 3.3× nutrient | 350.2 e | 255.55 gh |
| Uncompahgre River | 17.3 a | 32.32 abc |
| Uncompahgre River + 0.03× nutrient | 36.5 b | 51.82 b |
| Uncompahgre River + 0.3× nutrient | 79.0 c | 135.30 e |
| Uncompahgre River + 1.0× nutrient | 180.6 d | 292.38 h |
| Uncompahgre River + 3.3× nutrient | 454.7 f | 376.68 fghi |

[1] Alphabetical coding of the 5 percent confidence interval is for given productivity parameter only.
[2] Letters in common denote that mean productivity values do not differ significantly at the 5 percent level.

# APPENDIX E

## BOYSEN RESERVOIR AABT RESULTS

This report presents the results of the first AGP study conducted on water samples collected in Boysen Reservoir and at the outlet.

The results of the first AGP study of the waters from Boysen Reservoir are given in table E-1 and show that the natural waters, with no artificial nutrient addition, exhibit a very low algal growth. Algal production in these natural waters is less than the 0.03X nutrient level of the control series. Only with the highest levels of artificial nutrient addition in the natural waters and the control series is the algae production reasonably similar. Inhibition of algal growth is apparent in the natural reservoir waters and at the lower nutrient addition levels. It is not evident from the chemical analyses shown in table E-2 why this inhibition results. The results of this study are not consistent with field observations.

Table E-1.—*Results of AGP tests—Boysen Reservoir (May 1979). Mean values of three replications per test*

| Sample tested[1] | 21-day dry mass, g/m³ | 7-day count, $10^3$ cells/mL | 7-day pH | 14-day count, $10^3$ cells/mL | 14-day pH | 21-day count, $10^3$ cells/mL | 21-day pH |
|---|---|---|---|---|---|---|---|
| Distilled water + 0.03× nutrient | 1.5 | 13.6 | 7.35 | 55.0 | 6.55 | 58.6 | 6.53 |
| Distilled water + 0.3× nutrient | 26.5 | 85.2 | 8.80 | 212.7 | 7.95 | 249.0 | 7.62 |
| Distilled water + 1.0× nutrient | 97.1 | 168.8 | 9.70 | 679.6 | 9.67 | 891.9 | 8.74 |
| Distilled water + 3.3× nutrient | 71.8 | 107.1 | 10.30 | 340.4 | 9.47 | 729.1 | 7.56 |
| Boysen Reservoir | 3.6 | 5.8 | 8.80 | 10.7 | 8.39 | 7.7 | 8.45 |
| Boysen Reservoir outlet + 0.03× nutrient | 2.5 | 15.4 | 8.80 | 17.3 | 8.41 | 8.2 | 8.47 |
| Boysen Reservoir outlet + 0.3× nutrient | 29.7 | 77.2 | 9.10 | 70.8 | 8.45 | 69.3 | 8.49 |
| Boysen Reservoir outlet + 1.0× nutrient | 67.2 | 52.0 | 9.30 | 158.5 | 8.61 | 267.1 | 8.61 |
| Boysen Reservoir outlet + 3.3× nutrient | 104.2 | 85.8 | 10.90 | 271.9 | 7.3 | 481.0 | 9.43 |
| Boysen Reservoir lower 1/3 | 6.3 | 10.7 | 7.50 | 17.9 | 8.33 | 9.0 | 8.32 |
| Boysen Reservoir lower 1/3 + 0.3 × nutrient | 8.0 | 6.0 | 7.55 | 19.4 | 8.31 | 12.2 | 8.39 |
| Boysen Reservoir lower 1/3 + 1.0 × nutrient | 30.7 | 94.2 | 8.55 | 384.0 | 9.31 | 580.5 | 9.14 |
| Boysen Reservoir lower 1/3 + 3.3 × nutrient | 122.1 | 125.9 | 9.40 | 523.3 | 9.22 | 807.0 | 9.14 |

[1] All waters were autoclaved prior to algae culturing to remove endemic species, and enriched with the standard nutrient solution at the given concentration.

Table E-2.—*Chemical analyses of field waters—Boysen Reservoir*

| Parameter[1] | Boysen Reservoir outlet, mg/L | Boysen Reservoir lower 1/3, mg/L |
|---|---|---|
| TDS | 482.0 | 384.0 |
| Calcium | 59.2 | 44.8 |
| Magnesium | 18.5 | 13.7 |
| Sodium | 71.3 | 53.6 |
| Potassium | 3.13 | 2.74 |
| Carbonate | 7.20 | 8.10 |
| Bicarbonate | 164.0 | 118.0 |
| Sulfate | 195.0 | 147.0 |
| Chloride | 10.3 | 7.45 |
| Anions + cations | 529.0 | 395.0 |
| Copper | <0.0005 | <0.0005 |
| Lead | <0.001 | <0.001 |
| Iron | 0.05 | 0.03 |
| Manganese | 0.01 | 0.02 |
| Zinc | 0.0205 | 0.0245 |
| Conductivity $\mu$S/cm | 697.0 | 572.0 |
| pH | 8.70 | 8.90 |

[1] Analyses by "Standard Methods for Examination of Water and Wastewater," 14th Edition, APHA, AWWA, WPCF, 1975.

GPO 856 - 737