TECHNICAL ASSISTANCE REPORT

TO THE

STATE OF NEVADA
DEPARTMENT OF HEALTH, WELFARE AND REHABILITATION

Analysis of Algal Growth Potential and Possible Discharge Requirements for the Lower Colorado River

UNITED STATES DEPARTMENT OF THE INTERIOR
Federal Water Quality Administration
Pacific Southwest Region
San Francisco, California

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I. INTRODUCTION

This report is the result of a series of events related to the development of a regional comprehensive water quality control program for the metropolitan area of Las Vegas, Nevada. An Inter-Agency Water Pollution Control Task Force, composed of municipalities, utility districts, local industry, and agencies of local government, has developed several alternate water quality control plans for Las Vegas Valley. One of these plans provides for metropolitan waste water collection, treatment, and effluent discharge to the Colorado River below Hoover Dam. To adequately evaluate this plan, the Task Force requested the State of Nevada, Department of Health, Welfare, and Rehabilitation, to provide discharge requirements for the Lower Colorado River below Hoover Dam. With such requirements, the Task Force would then determine the degree of treatment necessary and whether or not the alternative plan is feasible.

The State of Nevada then requested the FWQA to provide technical assistance specifically for determining the effects of various nutrient loadings on the Colorado River and Lake Mohave and for consultation in the development of discharge requirements to the Lower Colorado River. The State of Nevada plans to begin official procedures for establishing the discharge requirements in July 1970.

The purpose of this study is to provide the State of Nevada with data on which to base nutrient discharge requirements for the Colorado River below Hoover Dam. Specifically, the study provides an understanding of the potential algal growth response of the receiving waters for the condition of direct discharge to the river.

This report is the preliminary phase of a two-phase project. The objective was to collect and analyze samples from the study area during February and March 1970, and to determine algal growth characteristics before seasonal instream algal growth began. The second phase will duplicate this initial phase in August 1970 to provide comparative results during the active algal growth season. Consequently, the conclusions and recommendations from this report should be considered preliminary subject to change on the basis of results from the second phase.

Authority

The Federal Water Quality Administration is the agency of the Federal government having primary responsibility for implementation of a national policy for enhancement of the quality of the nation's water resources through the control of pollution. This policy is described in the Federal Water Pollution Control Act, as amended (33 U.S.C. 466 et seq.). Section 5 of this Act authorizes the FWQA to provide technical assistance to appropriate public agencies for investigations and studies relating to the causes, control, and prevention of water pollution.

These studies were initiated by a letter of request from the State of Nevada, Department of Health, Welfare, and Rehabilitation, dated December 5, 1969. A copy is included in the report appendix.

Study Area

The study area is shown in Figure 1. It extends from the upper end of Boulder Basin in Lake Mead downstream to Davis Dam. Sampling stations were selected to reflect changes in water quality and algal growth characteristics between important geographical, hydrological, and structural features. These stations are described in Table 1.

Table 1. Description of Sampling Locations

Station No.	Description
1	Lake Mead upstream end of Boulder Canyon, across from lighted marker on state line.
2	Lake Mead in Boulder Canyon, across from Canyon Point on state line.
3	Lake Mead in Boulder Basin across from Callville Bay on state line.
4A	Las Vegas Bay 0.15 mile from mouth of Las Vegas Wash.
4	Las Vegas Bay one-third mile from Las Vegas Boat Harbor near center channel.
5	Las Vegas Bay about 2-1/2 miles from Las Vegas Boat Harbor toward Lake Mead.
6	Lake Mead at Hoover Dam.
7	Colorado River upstream from Willow Beach and Geological Survey gage at Cableway.
8	Colorado River at head of Windy Canyon six miles below Willow Beach.
9	Colorado River 200 yards below Davis Dam at mid-channel.
LVGW-1	Large gravel pit (NE 1/4, Sec 31, T21S, R63E) down-gradient of the BMI ponds on the south side of Las Vegas Wash.
LVGW-2	Hand auger hole (NW 1/4, Sec 31, T21S, R63E) immediately north of the upstream BMI ponds. This represents the quality of seepage which moves from the BMI ponds into Las Vegas Wash.
LVGW-3	Gravel pit (SE 1/4, Sec 35, T21S, R62E).
LVGW-4	Hand auger hole located between Duck Creek and Las Vegas Wash, along the section line of Sections 23 and 26, one-fourth mile east of the section corner in T21S, R62E.
LVGW-5	Outlet of Charleston Drain.

II. SUMMARY

Grab samples were collected on three occasions within the study area (January 25-26, February 16-17, March 1-4) and were analyzed for nitrogen, phosphorus, temperature, conductivity, and algal growth potential (AGP). AGP bioassays were conducted for control or background conditions and for conditions in which calculated amounts of nutrients were added to the samples. The forms of added nutrients were: (1) inorganic chemical nitrogen and phosphorus, (2) secondary sewage treatment plant effluent, and (3) near-surface groundwater inflow to Las Vegas Wash.

Findings

1. Grab samples collected from the surface waters of Lake Mead and the Colorado River below Hoover Dam had nitrate nitrogen (NO_3-N) concentrations between 0.30 and 0.66 mg/l and total soluble phosphorus concentrations of 0.006 to 0.054 mg/l. No significant difference was noted between nitrate results by location. However, the phosphorus concentration at Stations 4 and 5, near Las Vegas Bay, were significantly greater than those at upstream or downstream stations.

2. The results of the AGP bioassays on control samples reported as maximum growth response, indicate an average level of 11.5 μg/l at Stations 1 and 3 above the Las Vegas Wash, 30.3 μg/l at Stations 4 and 5 near the Las Vegas Wash, and between 17.1 to 21.6 at Stations 6, 7, and 8 below the Las Vegas Wash. A large number of replicate samples were analyzed to determine the reproducibility of the AGP tests at each of the eight stations at the 95 percent

confidence level. In nearly every case the maximum deviation noted was less than 10 percent.

3. AGP bioassays were conducted on Lake Mead and Colorado River samples seeded with inorganic nitrogen and phosphorus. Only those samples seeded with phosphorus created a growth response statistically different than the controls. The response was generally three to five times greater than that for the control samples.

4. AGP bioassays were conducted on samples seeded with secondary sewage treatment plant effluent, near surface groundwater and inorganic phosphorus. The purpose of the effluent seed was to simulate a direct waste discharge to the river. The groundwater seed simulated potential Las Vegas Wash flows without present waste flows. The inorganic phosphorus seed provides a base for comparison with this and other AGP bioassays.

It was found that the response to the groundwater seed was much greater and that the STP and KH_2PO_4 seeds were similar up to an addition of about 15 μg Phosphorus/1, after which the STP seeded sample gave a greater response.

5. The possibility of iron as a contributor to algal growth was also investigated in a set of AGP bioassays. It was found that by seeding control samples from Lake Mead with iron the algal growth doubled. However, no significant difference was noted between the AGP results on samples seeded with groundwater, STP effluent and KH_2PO_4 with iron added and those without additional iron.

Conclusions

The results of the investigations described in this report have led to the following tentative conclusions:

1. Under existing conditions, nitrogen does not limit the growth of algae in the waters of Lake Mead and the Colorado River below Hoover Dam.

2. Inorganic phosphorus is a limiting factor in the growth of algae in waters of Lake Mead and the Colorado River below Hoover Dam.

3. For existing quality conditions, the algal growth potential as measured by the AGP test increases between Lake Mead, upstream of influences from Las Vegas Wash inflow, and below Willow Beach in Lake Mojave.

4. A density current is created by the Las Vegas Wash influent into Lake Mead due to high concentrations of salt in the Wash flow. However, the extent and significance was not determined in this study. Delete

Recommendations

As a result of the above findings and conclusions, it is tentatively recommended that:

1. The State of Nevada informally establish a desired water quality goal for the Lower Colorado system which would prevent eutrophication nuisances.

2. The State of Nevada require that discharges of treated wastewater effluents to Lake Mead or below Hoover Dam be consistent with the desired water quality goals; i.e., the projected mix of receiving water and treated wastewater effluent shall not produce

statistically measurable biostimulation above the water quality goal.

3. The water quality goal established should be based on a combination of both the AGP test and total soluble phosphorus as defined below. Using a representative sample of properly mixed receiving water and treated wastewater effluent, the: (a) peak algal crop development in a 10-day incubation period, expressed as μg/l chlorophyll, be limited to 10-20μg/l. (b) The total soluble phosphorus be limited to 10-20μg/l as P.

III. STUDY PROCEDURES

The project was planned in two phases to evaluate water quality conditions in Lake Mead and the Colorado River below Hoover Dam before and during the period of peak seasonal algal growth. Phase I, the period before algal growth, was conducted during January through March 1970. Phase II will be conducted in August 1970 and will duplicate the procedures used in Phase I.

To provide an analysis of the receiving water's response to nutrient discharges, an algal bioassay technique termed the Algal Growth Potential (AGP) test was utilized. This test does not provide absolute indices of optimum nutrient concentrations desired for a particular waste discharge, but can indicate several important algal response characteristics such as:

(1) Whether or not specific nutrient elements are limiting to the growth of algae.

(2) The relative algal growth characteristics of a receiving water as influenced by various waste sources.

(3) The concentration of specific nutrients added to the receiving water at which algal growth begins or is accelerated.

In drawing conclusions from the test results, it must be recognized that the test is a laboratory analysis which is performed in simulated conditions which may not correspond precisely with the natural receiving water system.

Four independent AGP studies were performed on samples collected from the Colorado River between the upper reaches of Lake Mead and Davis Dam. The objectives of each of these tests were:

Bioassay #1 and 2: To determine if either phosphorus or nitrogen were limiting algal growth in the samples.

Bioassay #3: To determine whether the phosphorus in groundwater (collected between BMI ponds and Las Vegas Wash) and secondary STP effluent gave the same algal growth response as the addition of phosphorus in a chemical form (KH_2PO_4).

Bioassay #4: To determine if the presence of excess iron in groundwater was the cause which stimulated algal growth beyond that produced by the addition of secondary effluent and chemical phosphorus.

Sampling Program

Grab sampling procedures were used for the collection of all samples. A total of nine surface water sampling stations in Lake Mead and the Colorado River below Hoover Dam were established. These are described in Table 1 and shown in Figure 1. In addition, five groundwater sampling stations were established for near surface inflow to Las Vegas Wash. These are also shown on Figure 1 and described in Table 1.

The sampling program for this phase (Phase I), of the study included three separate surveys of the study area: January 25-26, February 16-17 and March 2-4, 1970. During the first survey, which was originally planned as a reconnaissance survey only, grab samples from the surface were collected once per day from stations (3,4,6,7, 8) as shown in Figure 1. Chemical analysis for the nitrogen and phosphorus forms, temperature and SEC were made for each sample and the AGP bioassay #1.

The second survey was a duplication of the first with an expansion of the number of surface water stations sampled to nine (Stations 1 - 9 as shown on Figure 1). In addition, depth samples were collected at stations 1, 2, and 6.

During the third survey, all nine of the surface water stations were sampled which, with the exception of stations 7, 8 and 9, included depth samples also. Near surface groundwater samples were collected at the five stations described in Table 1 and composited into one sample for use in AGP bioassays 3 and 4.

The composite sample was prepared from samples collected at the above five sites and had the following approximate volumetric composition:

Site	Percent of Total Sample
LVGW-1	30
LVGW-2	15
LVGW-3	15
LVGW-4	30
LVGW-5	10

Another site (LVGW-6) was sampled but was not used for the composite. This site represents seepage which forms the flow of Duck Creek above the Nevada Power Company plant.

The secondary STP seed used in AGP bioassays 3 and 4 was collected at the confluence of the Clark County and Las Vegas STP effluents.

The same chemical analyses were made on all samples as described in the first survey. AGP bioassay #3 was conducted for surface water samples collected during this survey and utilized the groundwater composite, STP sample and KH_2PO_4 as seeds.

AGP bioassay #4 was conducted using samples collected during the third survey after noting from bioassay #3 that the algal growth from groundwater was much greater than for the other two seeds.

Analytical Procedures

Field analyses were made for temperature and specific electrical conductance (SEC). Nitrogen and phosphate analyses were conducted at the Colorado River-Bonneville Basins Laboratory at Salt Lake City, Utah. Standard analytical procedures according to the 12th Edition of Standard Methods were utilized for both the field and laboratory procedures.

The AGP bioassays were conducted at the Southwest Regional Laboratory in Alameda, California. The AGP test is not presently considered a "standard test"; however, its use and procedures have been well refined. A general description of the AGP bioassay is given here. If specific procedural details are required, they can be obtained from the Southwest Regional Laboratory at Alameda, California.

Bioassay Methodology

Although the AGP analysis is a complex biological test which requires rigorous technical skills, the test is conceptually quite simple and straightforward. For the present application of the test, water under study - say, Lake Mead - is combined with nutrient sources (seed) in several replicate flasks and incubated under artificial light conditions until a maximum growth of algae is recorded. Flasks without added nutrients are also tested to provide a relative measure of algae response with existing background conditions. The nutrient seeds used in these studies were: nitrogen as KNO_3, phosphorus as KH_2PO_4, secondary sewage treatment plant effluent, near-surface groundwater collected between BMI ponds and Las Vegas Wash, and iron.

The results of the bioassay are given as (1) the maximum amount of chlorophyll reached in the sample, (2) the increase in chlorophyll derived by subtracting initial sample concentrations from maximum values, and (3) the maximum observed growth rate ($\hat{\mu}$, day^{-1}). Maximum growth (item 1) is the most common and useful measure of growth for these studies.

IV. RESULTS

A summary of all chemical analyses is presented in Table 2. Besides characterizing the waters used in the AGP tests, these data provide the basis for evaluating variations of quality with sample depth and geographical location. The most striking result is the relatively small variation in quality between stations. The ranges of nitrate and total phosphorus found in surface samples are tabulated below in related station groups:

Range of Results, mg/l

Stations	NO_3-N	Total Sol. P
1-3	0.36 - 0.66	0.007 - 0.020
4 & 5	0.32 - 0.46	0.020 - 0.054
6-9	0.30 - 0.64	0.006 - 0.023

The variation of temperature and SEC was extremely slight except for the case of samples taken close to the bottom of Las Vegas Bay at stations 4A and 4. These samples indicate that the Wash inflow maintains its integrity as a density current for considerable distance out into Las Vegas Bay. The extent and significance of this phenomenon will be investigated at a later time.

Results of the four AGP bioassays are presented in a graphical and tabular form and represent a condensation of detailed laboratory studies available as open-file reports.

Bioassay #1 & 2

Samples for these tests were collected on January 25-26, 1970 and February 16-17, 1970, with the January collection serving as a preliminary test. Samples were collected once for bioassay #1 at Stations 3, 4, 6, 7, and 8; and once for bioassay #2, at all stations 1 through 9. Each sample was divided into three parts. One part remained the control with no additions. To one of the other parts was added 0.10 mg P/1 (KH_2PO_4) and to the other, 0.50 mg N/1 (KNO_3). The chemicals added were concentrated so that only about a milliliter had to be added to a liter of the sample, thus only very slightly diluting the original water. The chemical analyses of water used in bioassay #1 are presented in Table 3.

Tables 4, 5, and 6 statistically describe the AGP results with respect to variations between stations, type of nutrient seed, and type of algal growth measurement. It is important to note in these tables that the values of growth response connected by underlining do not differ at the 95 percent confidence level.

Figure 2 shows a typical growth response observed during bioassays #1 and #2 incubation.

Table 2. Basic Chemical Results
Lake Mead - Colorado River Study

		Station Number	Date Collected (1970)	Sample Depth	Nitrate NO_3-N mg/1 as N	Ammonia NH_3-N mg/1 as N	Organic N mg/1 as N	Total -N Kjeldahl mg/1 as N	Phosphate Ortho mg/1 as P	Phosphate Tot-Sol mg/1 as P	Temp °C	Specific Electrical Conductance
I.	Lake Mead-Colorado River	3	1/26	S	0.56	0.03	0.21	0.24	0.003	0.020	14	1,300
		6	1/26	S	0.48	0.07	0.29	0.36	0.010	0.016	12	1,350
		7	1/26	S	0.51	0.03	0.34	0.37	0.013	0.020	13	1,350
		8	1/25	S	0.64	0.04	0.19	0.23	0.013	0.023	13	1,200
	Las Vegas Bay	4	1/26	S	0.41	0.04	0.10	0.14	0.007	0.020	12	1,400
II.	Lake Mead-Colorado River	1	2/17	S	0.66	0.07	0.23	0.30	0.002	0.007	14	1,250
		1	2/17	2/3 Depth	0.63	0.06	0.24	0.30	0.002	0.007	13	1,200
		2	2/17	S	0.48	0.05	0.33	0.38	0.003	0.10	13	1,300
		2	2/17	2/3 Depth	0.64	0.06	0.28	0.34	0.003	0.007	13	1,200
		3	2/17	S	0.36	0.07	0.28	0.35	0.007	0.016	13	1,320
		6	2/17	S	0.32	0.07	0.24	0.31	0.003	0.016	13	1,320
		6	2/17	104'	0.40	0.05	0.26	0.31	0.007	0.020	13	1,300
		6	2/17	254'	0.59	0.04	0.19	0.23	0.010	0.020	13	1,200
		7	2/16	S	0.37	0.06	0.23	0.29	0.007	0.016	14	1,200
		8	2/16	S	0.37	0.05	0.23	0.28	0.007	0.020	14	1,250
		9	2/16	S	0.30	0.04	0.23	0.27	0.003	0.013	12	1,300
	Las Vegas Bay	4	2/17	S	0.32	0.04	0.32	0.36	0.016	0.032	13	1,350
		5	2/17	S	0.34	0.03	0.25	0.28	0.007	0.020	13	1,320
III.	Lake Mead-Colorado River	1	3/2	S	0.47	0.05	0.31	0.36	0.007	0.016	13	1,280
		1	3/2	250'	0.61	0.05	0.23	0.28	0.004	0.009	12	1,250
		2	3/2	S	0.38	0.07	0.28	0.35	0.009	0.020	13	1,300
		2	3/2	250'	0.64	0.03	0.28	0.31	0.004	0.009	12	1,230
		3	3/2	S	0.52	0.07	0.21	0.28	0.006	0.016	13	1,300
		3	3/2	250'	0.54	0.04	0.26	0.30	0.008	0.015	12	1,300
		6	3/2	S	0.55	0.05	0.22	0.27	0.012	0.022	12	1,300
		6	3/2	104'	0.55	0.03	0.35	0.38	0.017	0.030	12	1,300
		6	3/2	254'	0.61	0.03	0.24	0.27	0.012	0.023	12	1,250
		7	3/3	S	0.62	0.01	0.24	0.25	0.012	0.015	13	1,260
		8	3/3	S	0.61	0.03	0.22	0.25	0.012	0.016	12	1,300
		9	3/4	S	0.40	0.03	0.23	0.26	0.005	0.006	12	1,350

Table 2. (Contd.) Basic Chemical Results
Lake Mead - Colorado River Study

	Station Number	Date Collected (1970)	Sample Depth	Nitrate NO_3-N mg/1 as N	Ammonia NH_3-N mg/1 as N	Organic N mg/1 as N	Total -N Kjeldahl mg/1 as N	Phosphate Ortho mg/1 as P	Phosphate Tot-Sol mg/1 as P	Temp °C	Specific Electrical Conductance
Las Vegas Bay	4A	3/4	S	0.17	0.40	0.38	0.78	0.138	0.184	14	1,400
	4A	3/4	0.5' above bottom D = 12'	6.30	0.68	0.48	1.16	3.260	4.000	14	5,100
	4	3/4	S	0.32	0.07	0.39	0.46	0.005	0.054	13	1,300
	4	3/4	3' above bottom D = 70'	≤0.02	0.19	0.34	0.53	0.820	1.010	13	2,200
	4	3/2	S	0.38	0.05	0.34	0.39	0.025	0.050	13	1,350
	5	3/2	S	0.46	0.03	0.33	0.36	0.009	0.025	13	1,300
	5	3/2	120'	0.49	0.06	0.26	0.32	0.017	0.033	12	1,350
Ground Water Characterization											
Composite of samples (LVGW 1-5)	--	3/2	--	9.80	0.05	--	0.60	--	0.160	--	--
Gravel Pit	LVGW-1	3/1	--	0.34	0.06	0.04	0.10	0.010	--	19	6,000
Seepage below BMI Ponds	LVGW-2	3/1	--	2.10	0.15	0.68	0.83	0.047	0.316	13	7,000
Gravel Pit	LVGW-3	3/1	--	0.06	0.04	0.62	0.66	0.035	0.069	14	6,250
Groundwater between Duck Creek and Wash	LVGW-4	3/1	--	≤0.02	0.04	0.31	0.35	0.078	0.345	13	10,000
Outlet of Charleston Drain	LVGW-5	3/1	--	0.70	0.04	0.27	0.31	0.140	--	18	3,000
Duck Creek above NPC	LVGW-6	3/4	--	≤0.02	0.02	0.24	0.26	0.031	0.033	12	7,500

Table 3. Chemical Analyses of Water Used in Bioassay #1

Station Number:	3	4	6	7	8
Date (1970):	1/26	1/26	1/26	1/26	1/25
mg/l, NO_3-N	0.56	0.41	0.48	0.51	0.64
mg/l, NO_2-N	0.001	0.001	0.001	0.001	0.001
mg/l, NH_3-N	0.03	0.04	0.07	0.03	0.04
mg/l, Total Org N	0.21	0.10	0.29	0.34	0.19
mg/l, Total Kjeldahl N	0.24	0.14	0.36	0.37	0.23
mg/l, Ortho PO_4	0.01	0.02	0.03	0.04	0.04
mg/l, Ortho PO_4-P*	0.003	0.006	0.010	0.013	0.013
mg/l, Total Soluble PO_4	0.06	0.06	0.05	0.06	0.07
Temperature, °C	14	12	12	13	13
Conductivity µmhos/cm	1300	1400	1350	1350	1200

*PO_4-P = PO_4 x 0.326

Table 4. Comparison of Growth Response Measurements, Bioassay #1

Increase in Chlorophyll, μg Chl a/1

3n 8	8n	3	7n	6n	7	6	4n	4	3p	6p	4p	7p	8p
1.3	2.1	2.8	6.0	6.1	7.8	9.3	12.5	14.7	25.8	37.0	41.0	43.7	66.8

Maximum Chlorophyll, μg Chl a/1

8	3n	8n	3	6n	7n	7	6	4n	4	3p	6p	7p	4p	8p
10.1	10.5	11.0	12.2	14.7	15.0	16.8	17.9	31.0	33.4	35.2	45.7	52.7	59.6	60.3

Growth Rate - μb, Day^{-1}

8	8n	3	3n	7	6	7n	6n	4n	3p	4	6p	8p	4p	7p
0.06	0.12	0.13	0.15	0.17	0.21	0.24	0.28	0.31	0.33	0.34	0.48	0.50	0.53	0.61

CODE: Small "n" or "p" following the numbers signifies the nitrogen or phosphorus additions.

NOTE: Values connected by underlining are not different from each other at the 95% confidence level.

Table 5. Growth Responses of Individual Station Samples, Bioassay #1

Growth Parameter	Station Location	Control	N Addition	P Addition
Maximum Chlorophyll μg Chl a/1	3	12.2	10.5	35.2
	4	33.4	31.0	59.6
	6	17.9	14.7	45.7
	7	16.8	15.0	52.7
	8	10.1	11.0	75.6
Increase in Chlorophyll μg Chl a/1	3	2.8	1.3	25.8
	4	14.7	12.5	41.0
	6	9.3	6.1	37.0
	7	7.8	6.0	43.7
	8	1.3	2.1	66.8
$\hat{\mu}_b$, Day^{-1}	3	0.13	0.15	0.33
	4	0.34	0.31	0.53
	6	0.21	0.28	0.48
	7	0.17	0.24	0.61
	8	0.06	0.12	0.50

NOTE: Values connected by underlining are not different from each other at the 95% confidence level.

Table 6. Statistical Comparison of Growth Responses, Bioassay #1

Growth Parameter	CONTROL				
	8	3	7	6	4
Maximum Chlorophyll μg Chl a/l	10.1	12.2	16.8	17.9	33.4
Increase in Chlorophyll μg Chl a/l	1.3	2.8	7.8	9.3	14.7
μb, Day^{-1}	0.06	0.13	0.17	0.21	0.31
	NO_3-N ADDITION				
	8	3	7	6	4
Maximum Chlorophyll μg Chl a/l	11.0	10.5	15.0	14.7	31.0
Increase in Chlorophyll μg Chl a/l	2.1	1.3	6.0	6.1	12.5
μb, Day^{-1}	0.12	0.15	0.24	0.28	0.31
	ORTHO PO_4-P ADDITION				
	3	6	7	4	8
Maximum Chlorophyll μg Chl a/l	35.2	45.7	52.7	59.6	60.3
Increase in Chlorophyll μg Chl a/l	25.8	37.0	43.7	41.0	66.8
μb, Day^{-1}	0.33	0.48	0.50	0.53	0.61

NOTE: Values connected by underlining are not different from each other at the 95% confidence level.

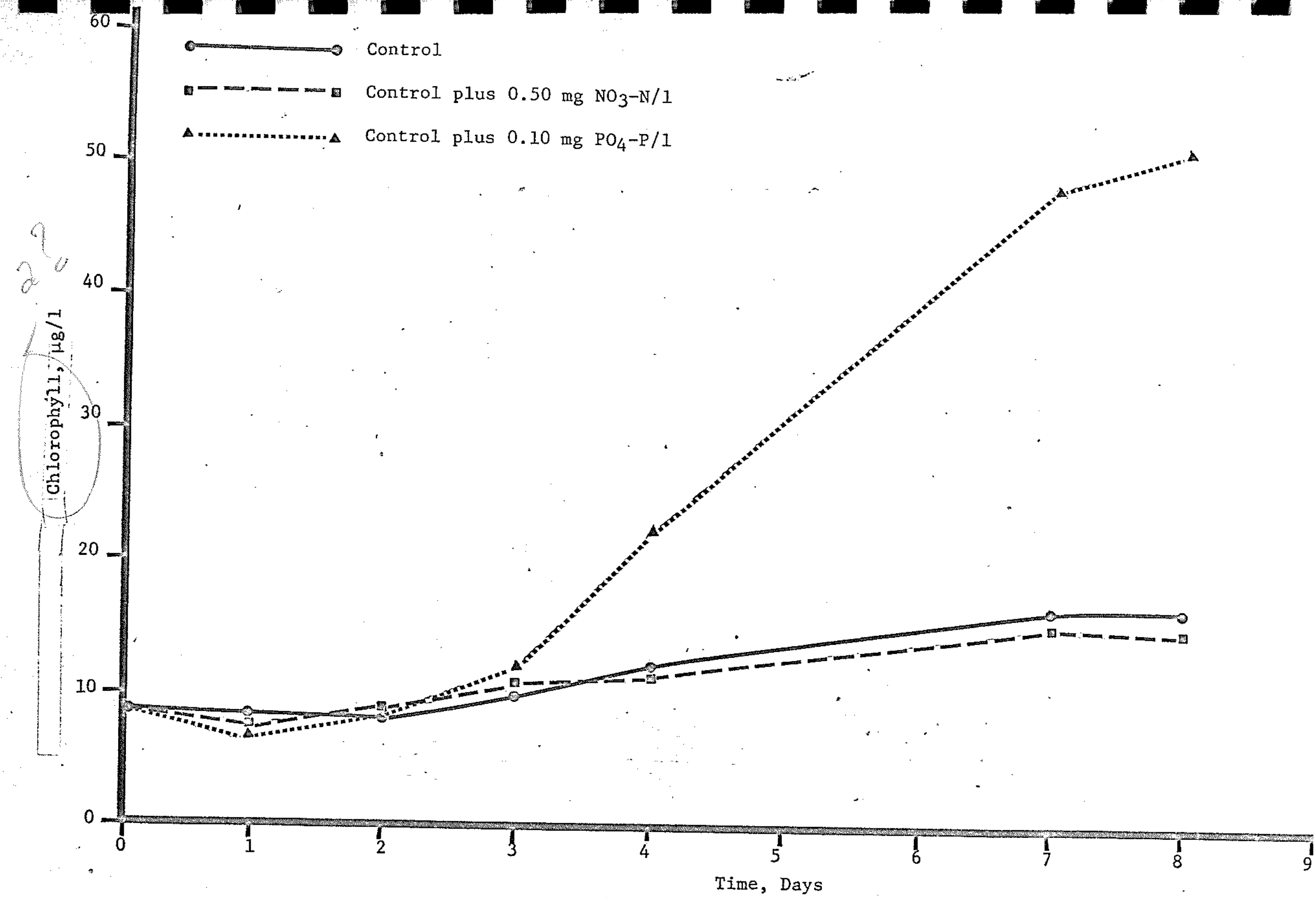

Figure 2. Typical Growth Response, Bioassay #1 and #2

The bioassay data, by all three measures of growth, gave similar results. The addition of phosphorus stimulated maximum response clearly greater than that observed in the control and in the samples to which nitrogen had been added. Growth response of the Station 3 samples, with phosphorus added, was lowest of all the phosphorus addition results. It is not statistically different from Station 4 control and nitrogen addition in the maximum chlorophyll comparisons,and overlaps several of the other waters in growth rate (μb) values. The Station 3 sample had the lowest phosphorus concentration (0.003 mg P/l) of all samples tested.

Although Station 3 phosphorus growth response was lower than those of the other phosphorus addition samples, the increase in chlorophyll above that of the control brought about by the PO_4-P additions gives additional information. Thus, there was approximately a three-to-six fold increase in chlorophyll concentrations over the controls for the Stations 6 and 7 samples and for the Station 4 sample. In contrast, Station 3 had a 20-times increase in chlorophyll concentration and Station 8, a 50-times increase.

It can be noted that not a single one of the nitrogen additions gave a response above the controls by any of the three growth measurements, and control samples from Stations 3 and 8 and nitrogen addition samples were consistently at the low end of the multiple-range test for all growth measurements. However, it may be significant to note that nitrogen additions only doubled the sample nitrate nitrogen concentrations, whereas the phosphorus additions were 10 times the amount of PO_4-P in the samples.

The chemical analyses of water used in bioassay #2 are presented in Table 7.

Table 7. Chemical Analyses of Water Used in Bioassay #2

Station Number	Nitrate NO_3-N mg/1 as N	Ammonia NH_3-N mg/1 as N	Organic N mg/1 as N	Total -N Kjeldahl mg/1 as N	Phosphate Ortho mg/1 as PO_4	Phosphate Ortho mg/1 as P	Phosphate Tot-Sol mg/1 as PO_4
1	0.66	0.07	0.23	0.30	0.007	0.002	0.02
2	0.48	0.05	0.33	0.38	0.01	0.003	0.03
3	0.36	0.07	0.28	0.35	0.02	0.006	0.05
4	0.32	0.04	0.32	0.36	0.05	0.016	0.10
5	0.34	0.03	0.25	0.28	0.02	0.006	0.06
6	0.32	0.07	0.24	0.31	0.01	0.003	0.05
7	0.37	0.06	0.23	0.29	0.02	0.006	0.05
8	0.37	0.05	0.23	0.28	0.02	0.006	0.06
9	0.30	0.04	0.23	0.27	0.01	0.003	0.04

NOTE: $P = PO_4 \times 0.326$.

Tables 8 and 9 statistically describe the AGP results from bioassay #2 with respect to variations between stations, type of nutrient seed, and type of algal growth measurement. The only additional work done in bioassay #2 was the counting of algal cells in two samples initially and at the end of growth. These counts are presented in Table 10.

The results of bioassay #2 were similar to those of bioassay #1. All samples with phosphorus seed had growth above both the control and the nitrogen seed when measured by maximum chlorophyll and total increase in chlorophyll. Growth rate data though did not show greater phosphorus responses for Stations 1, 2, 3, 5 and 9. This is attributed to the fact that it is usually more difficult to obtain differences in growth rates than in the other measured responses.

Responses to phosphorus addition were highest for the Willow Beach (Station 7 and 8) samples and lowest for the one below Davis Dam (Station 9). The sample from Willow Beach also gave the highest response to the phosphorus additions in the last experiment.

Bioassay #3

The chemical analyses of Lake Mead water used in this bioassay are presented in Table 11. The water was collected on March 2 from Lake Mead (Station 3 at the surface) and was tested for AGP using three seeds -- secondary STP effluent, composite groundwater sample, and inorganic phosphorus as KH_2PO_4. These seeds were added in amounts that would provide similar phosphorus concentrations so that a comparable relationship between algal growth response and phosphorus concentration could be developed for all three seeds. Table 12 presents the resultant nitrogen

Table 8. Growth Responses of Individual Station Samples, Bioassay #2

Growth Parameter	Station Location	Control	N Addition	P Addition
Maximum Chlorophyll μg Chl a/l	1	10.6	10.3	58.1
	2	10.5	9.8	37.3
	3	15.1	15.2	65.4
	4	24.8	30.5	67.5
	5	16.7	15.4	62.1
	6	18.9	16.8	48.4
	7	22.9	23.2	95.2
	8	33.8	33.5	97.9
	9	8.9	8.4	22.9
Increase in Chlorophyll μg Chl a/l	1	0.3	0.2	47.3
	2	0.0	0.2	25.7
	3	2.4	2.4	52.5
	4	6.4	12.1	49.0
	5	4.0	2.7	49.4
	6	5.4	3.3	34.9
	7	12.1	12.2	84.5
	8	19.8	19.4	84.0
	9	1.3	0.8	15.2
Growth Rate $\hat{\mu}_b$, day^{-1}	1	0.17	0.11	0.34
	2	0.03	0.03	0.24
	3	0.15	0.10	0.32
	4	0.15	0.14	0.45
	5	0.33	0.24	0.46
	6	0.18	0.06	0.48
	7	0.27	0.30	0.75
	8	0.50	0.45	0.80
	9	0.31	0.22	0.23

NOTE: Values connected by underlining are not different from each other at the 95% confidence level.

Table 9. Stations Compared for their Ortho Phosphorus Growth Responses, Bioassay #2

Growth Parameter	Phosphorus Growth Response								
	9	2	6	1	5	3	4	7	8
Maximum Chlorophyll μg Chl a/1	22.9	37.3	48.4	58.1	62.1	65.4	67.5	95.2	97.9
Increase in Chlorophyll	15.2	25.7	34.9	47.3	49.4	52.5	49.0	84.5	84.0
	9	2	3	1	4	5	6	7	8
Growth Rate $\hat{\mu}_b$, day^{-1}	0.23	0.24	0.32	0.34	0.45	0.46	0.48	0.75	0.80

NOTE: Values connected by underlining do not differ from each other at the 95% confidence level.

Table 10. Cell Count and Chlorophyll Concentration in the Phosphorus Spiked Samples Initially and at the End of Growth, Bioassay #2

Station	2/18/70 Initial		2/24/70 Terminal	
	Cells/ml	μg Chl a/l	Cells m/l	μg Chl a/l
1	2,150	10.7	12,130	57.7
4	4,030	12.6	20,830	61.8

Table 11. Chemical Analyses of Water Used in Bioassay #3

Location	Date	NH_3-N	Kjeld.* N	NO_2-N	NO_3-N	Ortho PO_4-P	Fe
Clark County and City of Las Vegas Sewage Effluent	3/2/70	13.1	20.0	0.18	0.59	8.2	0.094
Ground Water – A Composite of 5 Samples	3/2/70	0.05	0.60	0.01	9.8	0.16	0.11
Lake Mead (Station 3, Surface)	3/2/70	0.07	0.28	0.003	0.52	0.006	$< .02$

* Kjeld N = NH_3-N + Org N

Table 12. Total Inorganic Nitrogen and Ratios of N to P, Bioassay #3

LAKE MEAD WATER WITH ADDED INORGANIC PO_4-P					
Added PO_4-P μg P/1	0	5.0	15.0	30.0	50.0
Total PO_4-P in sample after addition of μg P/1	6	11.0	21.0	36.0	56.0
Total inorganic N in sample - μg N/1	583	583.0	583.0	583.0	583.0
Ratio of N to P	97	53.0	27.0	16.2	10.4
Percent of added seed volume to total incubated volume	Maximum was less than 0.5%				
LAKE MEAD WATER WITH ADDED SEWAGE EFFLUENT					
Added PO_4-P μg P/1	0	2.1	6.5	13.1	22.0
Total PO_4-P in sample after addition of μg P/1	6	8.1	12.5	19.1	28.0
Total inorganic N in sample - μg N/1	583	584.0	594.0	605.0	619.0
Ratio of N to P	97	72.0	47.0	33.0	22.0
Percent of added seed volume to total incubated volume	Maximum was less than 0.3%				
LAKE MEAD WATER WITH ADDED GROUND WATER					
Added PO_4-P μg P/1	0	2.1	6.5	13.1	22.0
Total PO_4-P in sample after addition of μg P/1	6	8.1	12.5	18.1	28.0
Total inorganic N in sample - μg N/1	583	720.0	995.0	1,405.0	1,963.0
Ratio of N to P in sample	97	89.0	80.0	78.0	70.0
Percent of added seed volume to total incubated volume	0%	1.4%	4.2%	8.3%	13.9%

and phosphorus data after the mixing of the samples with the three seeds.

The average growth response from bioassay #3, using the three types of seed (KH_2PO_4, STP and Groundwater) is presented in Table 13. This Table also indicates those values which do not differ from each other at the 95 percent confidence level. Table 14 presents algal cell counts and chlorophyll concentrations of two replicate Lake Mead control samples initially and at the end of incubation. While algae cell counts and chlorophyll both increased slightly, it is doubtful that the differences are statistically significant.

Figure 3 illustrates the growth response of Lake Mead water to the three nutrient seeds. Several features are readily apparent: (1) The response to the groundwater seed was immediate and much greater than for the other seeds and appears to be linear over the range studied. (2) The STP and KH_2PO_4 seeds responded similarly until about 15 μg P/l after which the STP seeded sample gave a greater response. (3) The KH_2PO_4 seeded sample appeared to level off at the higher phosphorus additions.

It is probable that the groundwater contained substances that stimulated immediate algal growth even at low phosphorus concentrations and promoted greater algal growth than occurred in the inorganic P and effluent additions at higher phosphorus levels. The possibility that this substance was iron was investigated in Bioassay #4.

<u>Bioassay #4</u>

The results of the bioassay are shown in Table 15 where the maximum chlorophyll concentration at the end of the experiment for the various

Table 13. Statistical Comparison of Individual Nutrient Seed Growth Response, Bioassay #3

μg P/1	0	5	15	30	50
KH_2PO_4 Maximum μg Chl a/1	11.9	12.4	15.9	18.2	18.9
μg P/1	0	2.1	6.5	13.1	22.0
Secondary effluent, Maximum μg Chl a/1	11.9	11.1	11.9	14.5	28.8
Ground water Maximum μg Chl a/1	11.9	15.0	19.9	26.7	40.8

NOTE: 1) Results - Average growth response from KH_2PO_4, STP, Ground water at various concentrations of phosphorus.
2) Values connected by underlining do not differ from each other at the 95% confidence level.

Table 14. Cell Count and Chlorophyll Concentration of Lake Mead Control Water, Bioassay #3

Replicate	3/2/70 Initial		3/11/70 Terminal	
	Cells/ml	μg Chl a/1	Cells/ml	μg Chl a/1
No. 1	2,090	10.5	3,460	11.7
No. 2	1,890	10.5	2,600	11.7

NOTE: The diatoms *Cylotella* and *Synedra* comprised 80% of the algal cells.

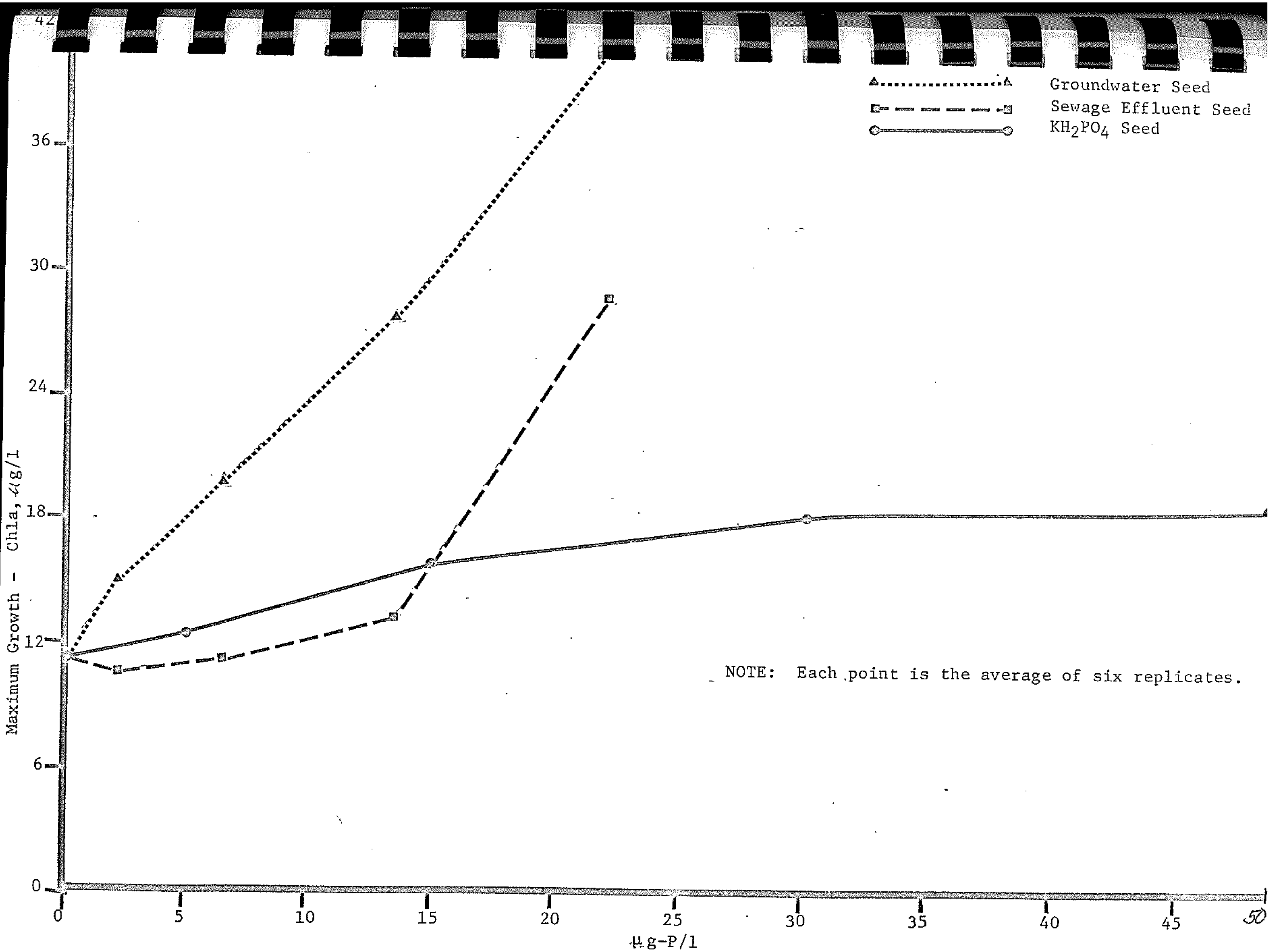

Figure 3. Growth Response of Lake Mead Water to Three Nutrient Sources, Bioassay #3

Table 15. Growth Response to Iron, Bioassay #4

Without Iron	With Iron								
LM-0	LM-0	LM-15	EF-15	LM-40 LM-80	GW-15	EF-80	EF-40	GW-40	GW-80
16.3	32.8	41.4	42.4	45.9	47.5	51.8	52.3	63.4	107.5

NOTE: 1) Maximum chlorophyll concentration of *Selenastrum capricornutum* in Lake Mead (LM) water without phosphorus additions, with 15, 40, and 80 μg P/1 added, and with effluent (EF) and ground water (GW) giving the same P concentration. Iron was added uniformly at 200 μg Fe/1.

2) Values connected by underlining do not differ from each other at the 95% confidence level.

concentration additions are statistically analyzed. These values are graphed in Figure 4, with further information on the samples given by the inorganic nitrogen content and nitrogen to phosphorus ratios of Table 16. The amount of iron in different volume additions of groundwater and effluent are shown in Table 17.

Groundwater still gave the greatest growth response in spite of additions of iron in excess to all samples (200 μg Fe/l). This was not true to the degree that it was in the bioassay #3. For example, at the 15 μg P/l level, groundwater responses were not statistically higher than those of the inorganic PO_4-P and sewage effluent samples (see Table 15). They were numerically higher, but the use of only two replicates might be responsible for the inability to distinguish between these values.

Groundwater responses were higher at the 40 μg P/l additions, but it is possible that the nitrogen could be limiting in the inorganic P and effluent samples.

The striking effect of the iron addition is on the Lake Mead controls, where the iron-spiked sample had twice the algal growth of the sample lacking iron.

V. DISCUSSION OF RESULTS

A key algal response characteristic identified by the AGP bioassay is whether or not a specific nutrient element limits algal growth. Biologists generally agree, that if the total inorganic nitrogen (NH_3-N + NO_3 - N) to Ortho Phosphorus (N to P) ratio in water exceeds the ratio found in algal cells living in the same water, then adequate nitrogen is present for algal growth as long as adequate phosphorus is also available. N to P ratios are in the neighborhood of 10:1 in

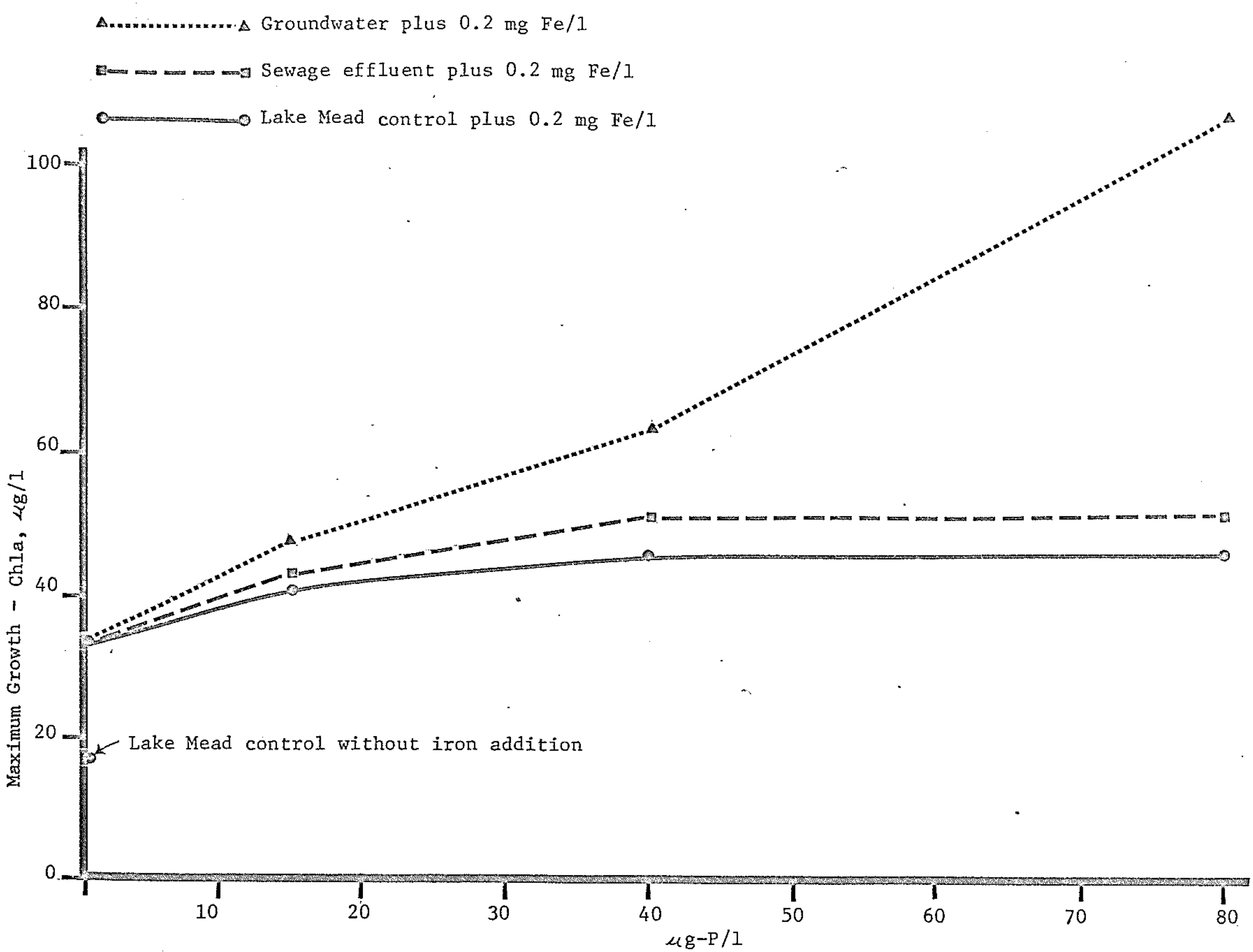

Figure 4. Growth Response of Lake Mead Water to Three Nutrient Sources Plus Iron, Bioassay #4

Table 16. Total Inorganic N and N to P Ratios, Bioassay #4

Added PO_4-P μg P/1		0	15	40	80
Total PO_4-P in Sample		6	21	46	86
Lake Mead with Added PO_4-P	Total Inorganic N in Sample μg N/1	583	583	583	583
	N to P Ratio	97	28	13	6.7
Sewage Effluent	Total Inorganic N in Sample μg N/1	583	608	651	718
	N to P Ratio	97	29	14	83
Ground Water	Total Inorganic N in Sample μg N/1	583	1,510	3,063	5,513
	N to P Ratio	97	72	67	64
	Percentage Addition to Lake Mead Water	0	9.4	25.0	50.0

Table 17. Iron Concentrations, Bioassay #4

Raw Sample Concentration

	Fe µg/l
Lake Mead	20
Sewage Effluent	94
Ground Water	110

Iron Added to Lake Mead Control

P Additions, µg-P/l	µg Fe/l Added	
	Ground Waters	Sewage Effluent
15	10.3	0.17
40	27.5	0.46
80	55.0	0.92

algal cells (Birdge and Juday, 1922). Therefore, by examining the N to P ratios and associated growth response data, it is possible to determine if nitrogen or phosphorus is limiting algal growth. For example, the ratio of N to P in bioassay #1 control samples ranged from 55:1 (Station 6) to 197:1 (Station 3). This shows an excess of nitrogen relative to phosphorus. When more nitrogen is added to the control no growth response is detected; but when phosphorus is added substantial growth results. Similar analysis for bioassays #2, 3, and 4 indicate that abundant nitrogen is available in the Lower Colorado River to support algal growth while available phosphorus may limit such growth.

It should be noted that for bioassays #3 and 4 (Tables 13 and 16) the N to P ratios fall into the range expected for algal cells for both the KH_2PO_4 and sewage effluent seeds at the higher concentration addition. Consequently, nitrogen may be limiting at these higher additions resulting in the apparent reduced growth shown in Figures 3 and 4.

Another important result of the AGP bioassays is an indication of the relative algal growth responses for existing background conditions between stations in the study area. An analysis of maximum growth for relevant station groupings follows.

Stations	Number of Replicates	Maximum Chlorophyll Response µg/l
1 - 3	32	11.5 ± 0.8
4 - 5	16	30.3 ± 3.0
6	24	17.1 ± 1.0
7 - 8	24	21.6 ± 3.8

The above table establishes quite well the existing levels of maximum growth.

These results also indicate that the inflow from Las Vegas Wash affects the quality of the Colorado River water. However, the amount of data available, including chemical quality, is not statistically strong enough to support such a firm conclusion.

Bioassay #3 was designed to evaluate the effect of two waste sources on the Colorado River as represented by upper Lake Mead water. Groundwater was used because if present waste flows are removed from the Wash, only groundwater will remain in the discharge to Las Vegas Bay. The secondary treatment plant effluent was used to simulate future advanced waste treatment discharges. Figure 3 describes the growth responses to these two sources if they are discharged in quantities capable of producing phosphorus concentrations up to 26 μg P/l.

It is apparent that groundwater (as defined by the composite sample) discharge to the Colorado River should be kept at a minimum to protect Las Vegas Bay from continued algal growth. The greater algal growth responses of groundwater may in part be due to its iron content. However, there are many inorganic micronutrients other than iron which could bring about growth differences, such as manganese, molybdenum, vanadium, cobalt, zinc, and boron. These might be contained in greater abundance in the groundwater than in Lake Mead water or in sewage effluent. Consequently, it would be difficult to establish which substances are responsible for the growth difference. It is doubtful that organic micronutrients are responsible for the differences because sewage effluent contains more of these substances than groundwater.

Accepting the results of bioassay #3 without qualifications would indicate that secondary waste effluent could be discharged until the instream phosphorus concentration reached about 18 μg-P/l without causing a measurable algal growth response. However, the following qualifications on such a conclusion are relevant.

(1) The secondary waste source probably does not adequately simulate future advanced waste treatment effluents.

(2) The results reflect only one bioassay. The bioassay will be repeated in August 1970.

VI. DISCUSSION OF DISCHARGE REQUIREMENTS

The development of effluent standards requires the following logical sequence of decisions: (1) determination of what beneficial uses are to be protected in the receiving waters, (2) establishment of quantitative criteria that will protect those uses, (3) calculation of effluent requirements that will meet the instream quality objectives for various conditions of discharge and river flow.

Since a determination of the beneficial uses to be protected has already been accomplished as a part of the water quality standards required by the Federal Clean Water Act of 1965, it remains only to complete the second and third steps described above to set effluent requirements.

Useful quantitative criteria may be expressed in terms of algal crops achieved or the nutrient concentrations which are found. However, the seasonal algal or aquatic plant nuisance achieved is probably the more direct criteria from the water quality control managers viewpoint since these are the eutrophication symptoms to which water uses react. A more detailed discussion of this follows.

Algal crops can be quantified in many ways ranging from visual evidences of turbidity, color or scum to more sophisticated measurements involving microscopy, fluorometry and spectroscopy. All have utility in defining water quality goals; however these discussions will be confined to the visual evidences and the chlorophyll content as derived from fluorometry measurements used in the AGP analysis.

Visual impacts can be related to more numerical measurements, at least crudely, and some judgements have been offered which relate turbidity and algal chlorophyll levels to recreational uses. Swimming safety has been considered borderline at secchi disc levels of 4 feet or less; nuisances due to blue green algae have been judged at a threshold when crops reach 50 μg/l in the Potomac estuary. Highly eutrophic lakes such as Clear Lake, California and Upper Klamath Lake, Oregon, commonly exceed 100 μg/l chlorophyll and are characteristically plagued with blue green algae, and most aesthetically offensive and least nourishing algae to aquatic animals. Oligotrophic lakes such as Lake Tahoe seldom generate chlorophyll levels in excess of 1 μg/l. Water clarity varies inversely with chlorophyll as shown in secchi disc values for Clear Lake which are commonly 5 feet or less and in Lake Tahoe where 100 foot readings are common.

The lower Colorado River, below Hoover Dam has demonstrated its abaility to grow sufficient algae for visual detection as evidenced by reports of "red tide" in Lake Mohave and complaints over diatom scums formed from periphyton growths below Davis Dam. As indicated by this study, laboratory incubation of Lake Mead and Colorado River waters generated about 20 μg/l chlorophyll. A comparison of chlorophyll values achieved in similar tests using California Basin waters is provided in Table 18 to put these recent tests in perspective.

TABLE 18

Algal Growth Potential Comparisons

Location	Season Sampled	AGP*
Kaweah River (East Fork)	Nov.	1.
Eel River below Scott Dam	Jun-Sept	2-5
American River below Nimbus Dam	July-Oct	4-6
San Joaquin Below Friant Dam	July-Oct	7-13
American River at mouth	July-Oct	26-34
Merced River at mouth	July-Oct	26-85
San Joaquin River at Vernalis	July-Oct	60-225
Klamath River near Klamath Falls	Nov	85-190
Stanislaus River at mouth	July-Oct	105-210
Tuolumne River at mouth	July-Oct	90-550

* Expressed as μg/l chlorophyll of peak algal crop developed in a 10 day incubation.

While the values tabulated suggest the lower Colorado River immediately below Hoover is not highly enriched, some caution is needed in view of the different seasons these waters were sampled. The visual impact of algal growths reported in the Lower Colorado suggest somewhat higher AGP values will occur in summer when more irrigation drainage waters enter the stream.

At this time the AGP test is perhaps the best parameter to use as a measure of stream biostimulation because the test reflects nitrogen, phosphorus, heavy metal, and other micronutrient effects. However, the AGP test does have characteristics which limit its use for routine surveillance, the worst being the length of time required for analysis. Consequently, the use of phosphorus would augment the AGP test by serving as a quicker analytical tool.

Since it is improbable that the states of Nevada, Arizona, and California will agree to compatible nutrient standards in time for use by the Inter-Agency Task Force, it is recommended that Nevada informally formulate its own instream quality objective on which to base effluent standards. A tentative instream quality objective utilizing both the AGP and phosphorus parameters is suggested below for the waters of Lake Mead and the Colorado River below Hoover Dam.

Using a representative sample of properly mixed receiving water and treated waste water effluent, the:

1. Peak algal crop development in a 10-day incubation period, expressed as μg/l chlorophyll, be limited to 10-20 μg/l.
2. The total soluble phosphorus be limited to 10-20μg/l as P.

These objectives are consistent with levels suggested by others for lakes such as Lake Erie, Lake Sebastacook, Maine, and Lakes in the Madison, Wisconsin area. However, bioassays of Colorado River waters which are considered acceptable during the summer season would be most helpful to support or adjust the above objectives.

A P P E N D I X

December 5, 1969

Paul DeFalco, Jr., Regional Director
Pacific Southwest Regional Office
Federal Water Pollution Control Admin.
U. S. Department of Interior
760 Market Street
San Francisco, California 94102

Dear Mr. DeFalco:

One of the proposals of the consulting engineers employed by the Interagency Task Force on the pollution control plan being developed for Las Vegas Valley is to provide additional treatment to the sewage effluents generated in the Valley and transport this effluent to a point near the intakes of Hoover Dam or to a point immediately below the Dam. The intent of this proposal is to avoid meeting those standards established by the Health Division for discharges to the Las Vegas Wash in the belief that lower standards would be acceptable for discharges to the main stream itself.

At the last Task Force meeting the chairman was directed to request the State Board of Health to develop standards for effluents being discharged to the main stream. As you can appreciate, such standards would pose many questions, the principal one being the effects of additional nutrient loadings comparable to those presently found in the River on a quiescent body such as Lake Mojave. Additionally, there is some question as to whether or not there would be assimilation or depletion of the nutrients through natural processes in the River between the Dam and Lake Mojave.

While we have not received the request from the Interagency Task Force, we would like to anticipate this request and have a reply ready as soon as possible to expedite implementation of the report being prepared for the Interagency Task Force.

We would appreciate any technical assistance your office may be able to provide in determining the effects of various nutrient loadings on the stream and Lake Mojave and consultation in development of the standards.

Very truly yours,

E. G. Gregory, Chief
Bureau of Environmental Health

EGG/jk