APPENDIX D

ALGAL GROWTH POTENIAL ASSAY (AGP)

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Algal Growth Potential (AGP) Assays

on

Water from the Rio Ruidoso

to

State Of New Mexico
Environment Department
1190 St. Francis Drive
P.O. Box 26110
Santa Fe, New Mexico 87502

submitted to

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by

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Background:

The water was collected on 5-20-02 and transported on ice to our laboratory. Water from each site was autoclaved and filtered, and used immediately. The initial tests for growth potential were initiated two days later and were terminated after 7 days of incubation under continuous illumination.

The procedures used for determining limiting nutrients and toxicity to algae was as established in the EPA-600/9-78-018 publication entitled "The *Selenastrum Capricornutum* Prinz Algal Assay Bottle Test" and EPA-660/3-75-034 publication entitled "Proceedings: Biostimulation/and/ Nutrient Assessment Workshop" The design is as follows:

Water from the creeks/rivers was autoclaved and passed through filters which had a pore diameter of 0.4 micrometers. The filtered water, 25 ml, was placed in 125 ml Erlenmeyer flasks which were cotton plugged. Each assay was conducted in triplicate.

The design of the test for algal growth potential are as listed below:

1. Control (filtered river water with no additions)
2. Control + 0.05 mg P/liter
3. Control + 1.00 mg N/liter
4. Control + 1.00 mg N + 0.05 mg P /liter
5. Control + 1.00 mg Na_2 EDTA/liter
6. Control + 1.00 mg Na_2 EDTA + 0.05 mg P/liter
7. Control + 1.00 mg Na_2 EDTA + 1.00 mg N/liter
8. Control + 1.00 mg Na_2 EDTA + 1.00 mg N + 0.05 mg P/liter
9. Control + 1.00 mg Na_2 EDTA + 1.00 mg N + 0.05 mg P + 4.5 µg Fe/liter

At the end of 7 days of incubation, the amount of chlorophyll was determined using fluoresence measurements. The fluorescence values were converted to dry weight values using a standard that we had constructed under these conditions of growth. The results are given in dry weight measurements as is accordance with the EPA procedure.

The water samples were designated as follows:

Designation	Site of collection
I	Rio Ruidoso @ Mescalero Boundary west of Ruidoso - upper canyon Road
II	Rio Ruidoso @ NM 267 ½ HWY 70
III	Rio Ruidoso above site on Susan Lattimer's property

Results:

The values for algal growth potential are given below as mg dry weight of algae/L.

Algal assays	Sites of water collection		
	I	II	III
1. Control (filtered river water with no additions)	0.108	0.695	0.086
2. Control + 0.05 mg P/liter	0.157	1.061	0.077
3. Control + 1.00 mg N/liter	0.528	1.856	0.274
4. Control + 1.00 mg N + 0.05 mg P /liter	0.742	1.268	0.421
5. Control + 1.00 mg Na_2 EDTA/liter	0.125	0.757	0.096
6. Control + 1.00 mg Na_2 EDTA + 0.05 mg P/liter	0.102	0.787	0.045
7. Control + 1.00 mg Na_2 EDTA + 1.00 mg N/liter	0.497	1.783	0.212
8. Control + 1.00 mg Na_2 EDTA + 1.00 mg N + 0.05 mg P/liter	0.718	1.380	0.421
9. Control + 1.00 mg Na_2 EDTA + 1.00 mg N + 0.05 mg P + 4.5 µg Fe/liter	0.853	1.554	0.779

A study concerning the effect of N and P additions on algal growth was conducted on appropriate creek/river waters. The growth values are presented below and as graphs for various additions of P and N alone.

Nutrients were added to the sterilized water and the amount of algal mass was determined after 7 days of incubation.

Productivity of algae as influenced by Nitrogen addition. Growth as mg dry weight/L.

Nitrogen added (Mg N/L)	Sites of water collection I	II	III
0	0.108	0.695	0.085
0.25	0.356	1.043	0.211
0.5	0.485	1.339	0.225
0.75	0.628	1.493	0.268
1.0	0.528	1.857	0.274
2.0	0.656	1.736	0.309

Productivity of algae as influenced by Phosphorus addition. Growth as mg dry weight/L.

Phosphorus added (Mg N/L)	Sites of water collection I	II	III
0	0.108	0.695	0.085
0.01	0.129	0.736	0.049
0.025	0.094	0.797	0.043
0.0375	0.092	0.849	0.053
0.05	0.157	1.061	0.262
.1	0.090	0.791	0.049

NOTE: Graphs of the N and P additions are in the attachment entitled graphs.

The following summary statements can be made concerning the individual waters:

Rio Ruidoso @ Mescalero Boundary west of Ruidoso - upper canyon Road

Site I has low algal productivity. Growth is increased by addition of nitrogen indicating that nitrogen is the primary limiting nutrient. When both nitrogen and phosphorus are added, a small increase in productivity occurs.

Rio Ruidoso @ NM 267 ½ HWY 70

Site II has a high level of algal productivity without nutrient additions. Growth was increased by nitrogen addition indicating that nitrogen is the limiting nutrient. The high phosphorous availability results in large increases in algal growth when the nitrogen levels are increased. Management procedures should not increase the amount of nitrogen entering the water at this site and if possible, the inputs of both phosphorus and nitrogen should be decreased.

Rio Ruidoso above site on Susan Lattimer's property

Site III has low algal productivity. Growth is increased by the addition of nitrogen and this indicates that nitrogen is the primary limiting nutrient. With added nitrogen, an increase in productivity occurs with additions of phosphorus. With addition of both nitrogen and phosphorus, a further increase in productivity occurs when iron is added.

Addition of the metal chelating agent, EDTA, to water from Sites I, II, and III did not increase algal productivity indicating that no toxic metals were inhibiting algal growth.

Comparison of Algal Growth Bioassay to Chemical Analysis of Water Samples

Results of the algal growth responses to nutrient additions are in agreement with the chemical analysis of water samples provided by the NM Environment Department. Sites I and II had very low algal growth potential without addition of nitrogen or phosphorus to the water samples as suggested by the very low dissolved nitrogen and total phosphorus present in the water samples. Nitrogen was the limiting nutrient for algal growth at both Site I and from Site II.

Chemical analysis of water samples from Site II showed nearly 10X as much dissolved nitrogen and nearly 20X as much total phosphorus as samples from Sites I and III. Without nutrient addition, algal growth was 7X greater in samples from Site II than from Sites I and III. Addition of nitrogen to samples from Site II resulted in further increases in algal growth; indicating that nitrogen is the limiting nutrient at Site II.

Aquatic plants identified from Rio Ruidoso

Site II	*Clasospora*, a green alga, was present. This organism is common throughout the state. It will grow as a dense mat in the water.
Site III	*Potamogeton foliosus* was present. This plant is commonly found in many of the water ways thorough out New Mexico.

Productivity

The basis for productivity classification of river water using standards established for lakes using the laboratory assay technique to assess biomass. (Reference: EPA-600/9-78-018 publication entitled "The *Selenastrum Capricornutum* Prinz Algal Assay Bottle Test" and EPA-660/3-75-034 publication entitled "Proceedings: Biostimulation/and/ Nutrient Assessment Workshop")

Classification	Algal cell density (algal dry weight)
Low productivity	0.00 - 0.10 mg/L
Moderate productivity	0.11 - 0.80 mg/L
Moderately high productivity	0.81 - 6.00 mg/L
High Productivity	6.10 - 20.00 mg/L

1. Status of water in Rio Ruidoso water at the three sites equivalent.

Site I (@ Mescalero Boundary)	Site II (Below the WWTP @ MM 267 ½)	Site III (@ Susan Lattimer's property)
Low productivity	Moderate productivity	Low productivity

2. Effect of N addition to the sites:

Site I (Mescalero boundary): Addition of 0.25 mg/L and 0.5 mg/L result in moving the trophic status up to the lower portion of the MODERATE level. The addition of 0.75mg/L and higher levels result in raising the productivity to the MODERATE level.

Site II (Below the WWTP): The addition of 0.25 mg N/L produces a MODERATELY HIGH level and addition of up to 2.0 mg/L of N produces a MODERATELY HIGH level.

Site III (Susan Lattimer's property): The addition of 0.25 - 2.0 mg/L of N gives MODERATE productivity.

3. Effect of P addition to the three sites:

Site I (Mescalero Boundary): Increase of P up to 0.1 mg/L does not increase cell yield and beyond 0.1 mg/L P the water is at the high end of LOW PRODUCTIVITY.

Site II (Below the WWTP) : Increase of P from 0.025 to 0.1 mg/L will trend toward MODERATELY HIGH productivity.

Site III (Susan Lattimer's property) : Addition of up to 0.1 mg/L of P will not exceed low productivity.

4. General comments:

- If limiting nutrient (typically nitrogen) is added then phosphorus addition will increase algal productivity.
- If phosphorus level is adequate to support algal growth but nitrogen is limiting, this creates a condition favorable for N_2 - fixing cyanobacterial growth.
- If phosphorus and nitrogen level is high enough to support algal growth, then green algae will be abundant. This is the case at Site II.

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