APPENDIX B
LIMITING NUTRIENT AND
ALGAL GROWTH POTENTIAL ASSAYS

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Algal Growth Potential (AGP) Assays

on

Water from the Rio Hondo

to

State Of New Mexico
Environment Department
1190 St. Francis Drive
P.O. Box 26110
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submitted to
Seva Joseph

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by

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Background:

The water was collected on March 31, 2004 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 that had a pore diameter of 0.4 micrometers. The filtered water, 25 ml, was placed in 125 ml Erlenmeyer flasks which were covered with aluminum foil. Each assay was conducted in triplicate.

The design of the test for algal growth potential is 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 Fe- EDTA/liter
6. Control + 1.00 mg Fe- EDTA + 0.05 mg P/liter
7. Control + 1.00 mg Fe- EDTA + 1.00 mg N/liter
8. Control + 1.00 mg Fe- EDTA + 1.00 mg N + 0.05 mg P/liter
9. Control + 0.0125 mg P/L
10. Control + 0.025 mg P/L
11. Control + 0.0375 mg P/L
12. Control + 0.100 mg P/L
13. Control + 0.25 mg N/L
14. Control + 0.50 mg N/L
15. Control + 0.75 mg N/L
16. Control + 2.00 mg N/L

At the end of 7 days of incubation, the amount of chlorophyll was determined using fluorescence 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 site of collection of the water samples was as designated below:

SITE	**DESIGNATION**
Taos Co. Rio Hondo above WWTP	I
Taos Co. Rio Hondo below WWTP	II
Taos Co. Rio Hondo at Rio Grande Confluence	III

Results:

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

Algal assays	Site of water collection		
	I	II	III
1. Control (filtered river water without additions)	0.603	0.922	0.826
2. Control + 0.05 mg P/liter	0.442	0.752	0.601
3. Control + 1.00 mg N/liter	1.515	0.861	1.078
4. Control + 1.00 mg N + 0.05 mg P /liter	1.527	1.644	1.462
5. Control + 1.00 mg Fe- EDTA/liter	0.517	0.996	0.773
6. Control + 1.00 mg Fe- EDTA + 0.05 mg P/liter	0.560	0.734	0.478
7. Control + 1.00 mg Fe- EDTA + 1.00 mg N/liter	1.581	0.662	0.863
8. Control + 1.00 mg Fe- EDTA + 1.00 mg N + 0.05 mg P/liter	1.507	1.789	1.754
9. Control + 0.0125 mg P/L	0.532	0.957	0.664
10. Control + 0.025 mg P/L	0.460	0.791	0.591
11. Control + 0.0375 mg P/L	0.329	0.943	0.552
12. Control + 0.10 mg P/L	0.429	0.578	0.525
13. Control + 0.25 mg N/L	1.118	0.941	1.153
14. Control + 0.50 mg N/L	1.249	0.986	1.174
15. Control + 0.75 mg N/L	1.411	0.892	1.135
16. Control + 2.00 mg N/L	1.411	0.997	1.028

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)	Site of water collection I	II	III
0	0.603	0.922	0.872
0.25	1.119	0.941	1.153
0.5	1.249	0.986	1.174
0.75	1.411	0.892	1.135
1.0	1.515	0.861	1.078
2.0	1.411	0.998	1.028

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

Phosphorus added (mg P/L)	Site of water collection I	II	III
0	0.603	0.922	0.872
0.0125	0.532	0.957	0.664
0.025	0.460	0.791	0.591
0.0375	0.329	0.943	0.552
0.05	0.442	0.753	0.601
0.10	0.429	0.579	0.525

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

The following summary statements can be made concerning the water:

Rio Hondo above WWTP (Site I) has moderate algal productivity. Growth is increased by nitrogen addition indicating that nitrogen is the limiting nutrient. Phosphorus addition does not increase algal growth either by itself or in combination with nitrogen addition.

Rio Hondo below WWTP (Site II) has moderately high algal productivity. Growth is increased by the addition of both nitrogen and phosphorus indicating that both are limiting for algal growth. Neither N nor P alone stimulated growth.

Rio Hondo at the Rio Grande (Site III) has moderately high algal productivity. Growth is increased by the addition of both nitrogen and phosphorus indicating that both are limiting for algal growth.

Productivity

The basis for productivity classification of river water is 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 Hondo water at the site tested equivalent to trophic status of lakes.

Site I - Moderate productivity

Site II - Moderately high productivity

Site III - Moderately high productivity

2. Effect of N addition to Site I:
Water from this site is nitrogen limited. Each addition of nitrogen increased productivity. Addition of 2.0 mg N/L raises the productivity to the lower portion of the MODERATELY HIGH PRODUCTIVITY range.

N addition to Site II:
Water at this site is both nitrogen and phosphorus limited. The addition of nitrogen to 1 mg or 2 mg/L raises the productivity only slightly because the water is also phosphorous limited. Thus, even with the addition of nitrogen to water from this site, the productivity level remains at the lower portion of the MODERATELY HIGH PRODUCTIVITY.

N addition to Site III:
Water from this site is both nitrogen and phosphorous limited. The addition of 1 mg or 2 mg/L raises the productivity only slightly because of the phosphorous limitation. Even with the addition of nitrogen to water from this site, the productivity level remains at the lower portion of the MODERATELY HIGH PRODUCTIVITY.

3. Effect of P addition to Site I:
This site is nitrogen limited and the addition of phosphorous does not increase the cell yield. When N addition was 1.0 mg/L and phosphorus was 0.05 mg/L the cell yield was substantially increased; however, this was attributed to the addition of N and not due to P. Addition of P from 0.0125 mg/L to 0.1 mg/L without the addition of N did not increase growth but, in fact, growth was slightly inhibited.

P addition to Site II:
This site is both N and P limited. The addition of phosphorous from 0.0125 mg/L to 0.1 mg/L did not increase growth yield but growth was slightly inhibited.

P addition to Site III:
This site is both N and P limited. The addition of phosphorous from 0.0125 mg/L to 0.1 mg/L did not increase growth yield but growth was slightly inhibited.

4. General comments:

- Without nutrient additions, the Rio Hondo has MODERATE PRODUCTIVITY to MODERATELY HIGH PRODUCTIVITY. With nitrogen and phosphorus additions, productivity increases but never exceeds MODERATELY HIGH PRODUCTIVITY. Management procedures should avoid any increase in nitrogen and phosphorus inputs.
- At Site I, nitrogen is limiting algal growth and with the addition of nitrogen up to 1 mg/L there is no limitation of growth due to phosphorus. If the limiting nutrients (nitrogen and phosphorus) are added to sites II or III, there is an increase in algal productivity. This indicates that Rio Hondo is limited for nitrogen but at sites II and III phosphorus is also limiting.
- If phosphorus level is adequate to support algal growth but nitrogen is limiting as is the case with site I, the condition favors N_2 - fixing cyanobacterial growth.

Chlorophyll a: The following are Chlorophyll a analysis on filter samples provided to us. We have used the procedure you had recommended and the calculations were by the following formula:

Chlorophyll a (mg/sample) =
[Corrected absorbance $_{665}$ x 28.66 x sample vol. x extractant vol] / [filtered sub-sample volume]

The extractant volume we used was 15 ml and the filtered sum-sample volume was the number of ml listed on the samples. That volume listed was 237 ml for Rio Hondo above WWTP, 253 ml for Rio Hondo below WWTP and 350 ml for Rio Hondo @ Rio Grande.

	Readings		Readings after acidification	
Sample sites	OD-665	OD-750	OD-665	OD-750
Rio Hondo above WWTP	0.194	0.009	0.118	0.011
	0.158	0.011	0.096	0.011
	0.188	0.016	0.114	0.013
ave =	0.180	0.012	0.109	0.012
Rio Hondo below WWTP	0.254	0.016	0.167	0.013
	0.245	0.012	0.163	0.015
	0.237	0.013	0.150	0.010
ave =	0.245	0.014	0.160	0.013
Rio Hondo @ Rio Grande	0.472	0.011	0.299	0.010
	0.486	0.014	0.304	0.012
	0.399	0.078	0.252	0.078
ave =	0.452	0.034	0.285	0.033

Site	Date collected	Chlorophyll a (mg/sample)
Rio Hondo above WWTP	03/31/04	**32.15 mg**
Rio Hondo below WWTP	03/31/04	**40.61 mg**
Rio Hondo @ Rio Grande	03/31/04	**111.01 mg**

Ash Free Dry Mass (AFDM) Rio Hondo

Sample	Date	Sample vol.(ml)	Sub-sample vol (ml)	AFDM /Sample* (g /sample)
Rio Hondo above WWTP	03/31/04	237	15	0.0537
				0.0553
				0.0537
			Mean ± SD =	**0.0542 ± 0.0009**
Rio Hondo below WWTP	03/31/04	253	15	0.0776
				0.0658
				0.0590
			Mean ± SD =	**0.0675 ± 0.0094**
Rio Hondo @ Rio Grande	03/31/04	350	15	0.1423
				0.1586
				0.1446
			Mean ± SD =	**0.1485 ± 0.0088**

SD = Standard deviation from the means.
* Formula for calculation: Ash-free dry mass (g per sample) =
[{(weight of crucible + filter + sample after drying) - (weight of crucible + filter + sample after ashing)} x sample volume] / [volume of filtered sub-sample]

Volume of filtered sub-sample was taken from label on each sample.

Summary of Chlorophyll a and AFDM:

- The triplicate values obtained for chlorophyll a and AFDM were very close
- As Chlorophyll a values for the sample increased, the AFDM values increased proportionally.