APPENDIX A
ALGAL GROWTH POTENTIAL (AGP) ASSAYS

Algal Growth Potential (AGP) Assays

on

Water from the Animas River

to

State Of New Mexico
Environment Department
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submitted to
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June 10, 2004

by

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Background:

The water was collected on April 23, 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 measurement. 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
Animas River @ Flora Vista	I
Animas River @ Aztec	II

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
1. Control (filtered river water without additions)	0.589	0.618
2. Control + 0.05 mg P/liter	0.601	0.624
3. Control + 1.00 mg N/liter	0.899	0.501
4. Control + 1.00 mg N + 0.05 mg P /liter	0.779	0.820
5. Control + 1.00 mg Fe. EDTA/liter	0.616	0.386
6. Control + 1.00 mg Fe- EDTA + 0.05 mg P/liter	0.491	0.286
7. Control + 1.00 mg Fe- EDTA + 1.00 mg N/liter	1.098	0.341
8. Control + 1.00 mg Fe- EDTA + 1.00 mg N + 0.05 mg P/liter	1.470	1.313
9. Control + 0.0125 mgP/L	0.433	0.525
10. Control + 0.025 mg P/L	0.433	0.439
11. Control + 0.0375 mg P/L	0.493	0.374
12. Control + 0.10 mg P/L	0.552	0.348
13. Control + 0.25 mg N/L	0.622	0.431
14. Control + 0.50 mg N/L	0.855	0.462
15. Control + 0.75 mg N/L	0.748	0.438
16. Control + 2.00 mg N/L	0.779	0.476

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	Site of water collection II
0	0.589	0.617
0.25	0.622	0.431
0.5	0.855	0.462
0.75	0.748	0.438
1.0	0.899	0.501
2.0	0.779	0.476

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

Phosphorus added (mg P/L)	Site of water collection I	Site of water collection II
0	0.589	0.617
0.0125	0.433	0.525
0.025	0.433	0.439
0.0375	0.493	0.374
0.05	0.601	0.624
0.10	0.552	0.348

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

The following summary statements can be made concerning the water:

Animas River @ Flora Vista (Site I) has moderate algal productivity. Growth is increased slightly by nitrogen addition indicating that nitrogen is one of the limiting nutrients. Phosphorus addition does not increase algal growth by itself but does increase growth when added along with nitrogen addition. It should be noted that the addition of iron along with both N and P accounts for greatest stimulation of growth indicating that iron is also a limiting nutrient in this river water.

Animas River @ Aztec (Site II) has moderate algal productivity. Growth is not increased by the addition of either nitrogen or phosphorus when added alone. When both N and P are added, growth is stimulated indicating that both are limiting for algal growth. It should be noted that the addition of iron along with both N and P accounts for greatest stimulation of growth indicating that iron is also a limiting nutrient in this river water.

Productivity

The basis for productivity classification of river water are 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 Animas River water at the site tested equivalent to trophic status of lakes.

Site I - Moderate productivity

Site II - Moderate productivity

2. Effect of N addition to Site I:

Water from this site is nitrogen and phosphorous limited. Addition of nitrogen (1 mg/L) or phosphorous (0.1 mg/L) did not increase growth when added individually. However, when added together productivity increased to the lower level of MODERATELY HIGH PRODUCTIVITY

range. Even with maximum stimulation of growth by the addition of N, P and Fe as Fe-EDTA, growth was at the lower level of MODERATELY HIGH PRODUCTIVITY.

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 and phosphorous t 0.05 or 0.1 mg/L, the productivity increased only to the lower level of MODERATELY HIGH PRODUCTIVITY. Even with the addition of Fe along with P and N additions, algal growth remained at the lower portion of the MODERATELY HIGH PRODUCTIVITY.

3. Effect of P addition to Site I:
This site is nitrogen limited and the singular 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 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. . Even with the addition of Fe along with P and N additions, algal growth remained at the lower portion of the MODERATELY HIGH PRODUCTIVITY.

P addition to Site II:
This site is both N and P limited. The singular addition of phosphorous from 0.0125 mg/L to 0.1 mg/L did not increase growth yield but growth was slightly inhibited. . Even with the addition of Fe along with P and N additions, algal growth remained at the lower portion of the MODERATELY HIGH PRODUCTIVITY.

4. General comments:

- Without nutrient additions, the Animas River has MODERATE PRODUCTIVITY. With nitrogen and phosphorus additions, productivity increases but never exceeds the lower range of MODERATELY HIGH PRODUCTIVITY. Singular additions of P or N to the level tested would not increase algal productivity. However, **management procedures should prevent the addition of <u>both</u> P and N to the Animas River**.

- Both sites of the Animas River were limited by iron for algal growth. This may be a pH effect and with runoff into the river becoming more acidic, growth of algae will increase. However, even with the addition of iron salts, **both sites are limited for both N and P**.

5. No samples were taken for chlorophyll a or ash free dry mass.