January 1979

Algal Bioassay Study for the Animas - La Plata Project

Leslie G. Terry
V. Dean Adams

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ALGAL BIOASSAY STUDY
for the
ANIMAS - LA PLATA PROJECT

By Leslie G. Terry and V. Dean Adams

Utah Water Research Laboratory
College of Engineering
Utah State University
Logan, Utah 84321

June 1979
ALGAL BIOASSAY STUDY FOR THE ANIMAS

LAPLATA PROJECT

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This report was completed for the United States Bureau of Reclamation as part of Contract No. 7-07-40-50329 (Chemical and Biological Analysis of Colorado Water Samples).

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LIMITING NUTRIENT BIOASSAYS

Sample Pretreatment:

The pretreatment procedure was the same for each of the five sample dates (Sept. 8, 1977; Nov. 29, 1977; Jan. 9, 1978; March 8, 1978; May 10, 1978). Immediately on arrival three liters of each sample was filter sterilized using 0.45 μ Millipore filters. Filtering removes native algae from the test water and enables the use of unialgal test species in the bioassay. Following filtration, the samples were subjected to routine chemical analyses for the determination of indigenous levels of soluble total and ortho phosphorus and soluble total inorganic nitrogen (Table III-1).

Chemical analysis is useful for identifying specific ions but cannot distinguish between biologically available ions and those which are not available. This is where the value of the bioassay lies. Bioassays use the measurable response of living organisms to environmental variables including determining whether or not nutrients are biologically available.

Experiment Set-Up Procedure:

The bioassays were conducted using 100 ml. sample volumes in 500 ml. Erlenmeyer flasks. Inverted beakers were chosen for flask closures in order to permit good CO₂-O₂ exchange and to prevent contamination.

Prior to use in the bioassays all glass and labware contacting the samples were treated in the following manner: sodium bicarbonate wash, tap water rinses, 1:1 hydrochloric acid rinses, deionized water rinses and finally ultra pure dionized water rinses. Following washing, all glassware was autoclaved using an aluminum foil closure at 121°C for 15 minutes.
Table III-1.
Animas-La Plata Project
Results of Chemical Analyses

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Orthophosphate (PO₄-P) (µg/l)</th>
<th>Total Soluble Phosphorus (µg/l)</th>
<th>Ammonia (NH₃-N) (µg/l)</th>
<th>Nitrate-Nitrite (NO₃+NO₂-N) (µg/l)</th>
<th>Nitrogen/Phosphorus* (NH₃+PO₄-P) N/P</th>
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</thead>
<tbody>
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<td></td>
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<td>16.</td>
<td>16.</td>
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<td>9.</td>
<td>45.</td>
<td>150.</td>
<td>195.</td>
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<td>2.</td>
<td>13.</td>
<td>66.</td>
<td>630.</td>
<td>348.</td>
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<td>2.</td>
<td>30.</td>
<td>44.</td>
<td>154.</td>
<td>99.</td>
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<td>69.</td>
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<td>19.</td>
<td>22.</td>
<td>54.</td>
<td>360.</td>
<td>22.</td>
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</tbody>
</table>

*A nitrogen/phosphorus ratio of <15 indicates nitrogen limitation while an N/P ratio of >15 indicates phosphorus limitation.
Samples from the Animas-La Plata project received the treatments listed on Table III-2 for each of the five sampling dates. Each treatment was set up in triplicate. The samples blanks (treatment A and H) were included to provide the basis for comparison of the other treatments and provide a measure of the general fertility of the sample. The control treatments were included to provide an estimate of theoretical cell growth and an index for comparing growth levels in the test waters.

Table III-3 lists the constituents of Algal Assay Medium (AAM). AAM is a precisely prepared growth medium containing known concentrations of all compounds essential to algal growth. The samples and control (with the exception of 9/8/77 controls) contained one half AAM levels of nitrogen and phosphorus whereas all other constituent were added at full strength levels. Di-sodium EDTA (Ethylene dinitrilo tetraacetic acid) a commonly used organic chelator, was added to Treatments A-G at a level of 1 mg/l in order to render excess metal ions biologically inactive. Serious metal toxicity was detected in earlier bioassays from the area. Treatment H and I (without EDTA) were included to confirm the metal toxicity. Increased growth in EDTA spiked samples in comparison to yields in the untreated flasks can be directly attributable to organic chelation and consequently metal toxicity.

Algal bioassays were performed according to EPA (1971) using the Green Alga, *Selenastrum capricornutum* PRINTZ. The test flasks were placed in a constant temperature room (24° ± 2°C) with "cool white" fluorescent lighting providing illumination of 400 ft-C (4304 lux)±10 percent.

The algal assays were monitored by determining the optical density (OD, Bausch and Lomb spectrophotometer to 750 nm, 1 cm path length) and
<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in NAAM</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compound mg/l</td>
<td>Element mg/l</td>
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<tr>
<td>A₁</td>
<td>NaNO₃</td>
<td>N 4.2</td>
</tr>
<tr>
<td>A₂</td>
<td>MgCl₂ 6H₂O</td>
<td>Mg 2.9</td>
</tr>
<tr>
<td>A₃</td>
<td>MgSO₄ 7H₂O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CaCl₂ 2H₂O</td>
<td>Ca 1.2</td>
</tr>
<tr>
<td>A₄</td>
<td>NaHCO₃</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>K₂HPO₄</td>
<td>P 0.186</td>
</tr>
<tr>
<td></td>
<td></td>
<td>μg/l</td>
</tr>
<tr>
<td>C</td>
<td>H₃BO₃</td>
<td>B 32.45</td>
</tr>
<tr>
<td></td>
<td>MnCl₂ 4H₂O</td>
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</tr>
<tr>
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<td>ZnCl₂</td>
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<td></td>
<td>Na₃MoO₄ 2H₂O</td>
<td>Mo 2.88</td>
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<tr>
<td></td>
<td>CoCl₂ 6H₂O</td>
<td>Co 0.35</td>
</tr>
<tr>
<td></td>
<td>CuCl₂ 2H₂O</td>
<td>Cu 0.004</td>
</tr>
<tr>
<td>D</td>
<td>FeCl₃ 6H₂O</td>
<td>Fe 33.05</td>
</tr>
<tr>
<td></td>
<td>Na₂EDTA 2H₂O</td>
<td>mg/l</td>
</tr>
</tbody>
</table>

Protocol for Nutrient Spiking

A₁ Nitrogen
B Phosphorus
A₁ + B N + P
C + D Trace Elements (T. E.)
ALL NAAM

Table III-2.
Animas - La Plata Project
Treatment Constituents

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Sample Treatments</th>
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</thead>
<tbody>
<tr>
<td>9/8/77</td>
<td></td>
<td>A. Sample + 1 mg/l EDTA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Sample + 1 mg/l EDTA + 2.1 mg/l Nitrogen (N)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Sample + 1 mg/l EDTA + 0.09 mg/l Phosphorus (P)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. Sample + 1 mg/l EDTA + Trace Elements (AAM Levels)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F. Sample + 1 mg/l EDTA + 15.0 mg/l NaHCO₃</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P + AAM levels of:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. Sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I. Sample + 2.1 mg/l N + 0.093 mg/l P + AAM levels of:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: Distilled water + 4.2 mg/l N + 0.186 mg/l P + AAM levels of:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control + EDTA: Distilled water + 1 mg/l EDTA + same as control above</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Sample Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/29/77</td>
<td></td>
<td>Control: Distilled Water + 2.1 mg/l N + 0.093 mg/l P + AAM levels of:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control + EDTA: Distilled Water + 1 mg/l EDTA + same as control above</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Sample Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/9/78</td>
<td></td>
<td>Control: Distilled Water + 2.1 mg/l N + 0.093 mg/l P + AAM levels of:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control + EDTA: Distilled Water + 1 mg/l EDTA + same as control above</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Sample Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/8/78</td>
<td></td>
<td>Control: Distilled Water + 2.1 mg/l N + 0.093 mg/l P + AAM levels of:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control + EDTA: Distilled Water + 1 mg/l EDTA + same as control above</td>
</tr>
</tbody>
</table>
Table III-2.
Animas - La Plata Project
Treatment Constituents

5/10/78
Animas River at Durango
Animas River at 32nd St. Bridge

Sample Treatments and Controls same as 11/29/77

La Plata River at Colo./N.M. Border
A. Sample
B. Sample + 2.1 mg/l N
C. Sample + 0.093 mg/l P
D. Sample + 2.1 mg/l N + 0.093 mg/l P
E. Sample + trace elements (AAM levels)
F. Sample + 15.0 mg/l NaHCO₃
G. Sample + 2.1 mg/l N + 0.093 mg/l P + AAM levels of:
   trace elements, NaHCO₃, CaCl₂, and MgSO₄

Control: same as 11/29/77
relative fluorescence (RF x 30, Turner Fluorometer, Model 110). Optical
density was measured over a 14 day period while relative fluorescence
was measured to monitor the progress of the cultures for the first six
to seven days when optical density does not provide a great deal of sensit­
ity. Fluorescence is a physiological response measuring chlorophyll
a and optical density is a measurement of biomass. Although they are
different measurements, the two can be correlated. Normally when chloro­
phyll a is increasing so is biomass and vice versa. The results of both
determinations are represented graphically in Figure 1-54. Maximum values
for optical density are listed on Table III-4.

Optical density (OD) is an indirect means of measuring algal cell
biomass. As a consequence OD is linearly related to biomass as dry
weight (Porcella et al., 1973). Due to this linearity biomass, as volatile
suspended solids (V.S.S) can be calculated directly from OD. The relation­
ship used to convert OD to V.S.S. in Table III-5 is:

\[ \text{V.S.S., mg/l} = 350 \times \text{OD} + 3.5 \]

Because of the difficulty of measuring biomass in low density cultures,
relative fluorescence of in v i o o chlorophyll a was used to estimate
biomass in the early phases of the bioassay. Maximum values for relative
fluorescence are listed on Table III-6. Calculations of average maximum
specific growth rate batch (\( \mu_b \)) were made using relative fluorescence.
The maximum specific growth rate occurs during the logarithmic phase of
growth; usually between day 0 and day 5. Maximum specific growth rates
are calculated on Table III-7.
Table III-4
Animas - La Plata Project
Maximum Amount of Growth Observed as Optical Density; 750 mm., 1 cm.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment</th>
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<td>A</td>
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<td>.002</td>
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<td>.005</td>
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</tr>
<tr>
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</table>
Table III-4.
Animas - La Plata Project
Maximum Amount of Growth Observed as Optical Density; 750 mm., 1 cm.

<table>
<thead>
<tr>
<th>Sample</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
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</tr>
<tr>
<td>Animas River at Durango</td>
<td>0.006</td>
<td>0.006</td>
<td>0.005</td>
<td>0.259</td>
<td>0.007</td>
<td>0.007</td>
<td>0.240</td>
<td>0.015</td>
<td>0.089</td>
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<tr>
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<td>0.007</td>
<td>0.025</td>
<td>0.285</td>
<td>0.009</td>
<td>0.007</td>
<td>0.305</td>
<td>0.015</td>
<td>0.064</td>
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<td>La Plata River near Colo./N.M. Border</td>
<td>0.048</td>
<td>0.080</td>
<td>0.061</td>
<td>0.250</td>
<td>0.068</td>
<td>0.049</td>
<td>0.340</td>
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Animas - La Plata Project
Maximum Amount of Growth Observed as mg/l VSS. a

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Table III-5.  
**Animas - La Plata Project**  
Maximum Amount of Growth Observed as mg/l VSS.$^a$

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$^a$VSS = Volatile Suspended Solids  
VSS, mg/l = 350 (Optical Density) + 3.5 (Porcella, et al., 1973)
### Table III-6.
**Animas-La Plata Project**
**Maximum Amount of Growth Observed as Relative Fluorescence, RF x 30**

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Table III-7.
Animas - La Plata Project
Maximum Specific Growth Rate; \( \hat{\mu}_b \), days\(^{-1} \)

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<th>F</th>
<th>G</th>
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</table>
| 9/8/77
| Animas River at Durango                                               | 0.12| 0.20| 1.54| 1.77| 0.09| 0.11| 1.75| 0.12| 0.10|
| Animas River at 32nd St. Bridge                                        | 0.11| 0.25| 1.87| 1.62| 0.12| 0.36| 1.82| 0.34| 1.65|
| Control                                                               |     |     |     |     |     |     |     |     | 1.87|
| Control + EDTA                                                        |     |     |     |     |     |     |     |     | 1.96|
| 11/29/77
| Animas River at Durango                                               | 0.41| 0.47| 0.63| 2.71| 0.20| 0.28| 2.15| 0.18| 0.99|
| Animas River at 32nd St. Bridge                                        | 0.62| 0.25| 0.27| 1.55| 0.24| 0.29| 1.25| 0.28| 0.25|
| La Plata River at Colo./N.M. Border                                   | 0.28| 0.25| 1.06| 1.12| 0.24| 0.26| 1.79| 0.13| 2.51|
| Control                                                               |     |     |     |     |     |     | 2.45|     |     |
| Control + EDTA                                                        |     |     |     |     |     |     |     |     | 2.83|
| 1/9/78
| Animas River at Durango                                               | 0.27| 0.69| 1.37| 1.65|     |     |     | 2.24| 0.11|
| Animas River at 32nd St. Bridge                                        | 0.06| 0.35| 1.02| 1.37|     |     |     | 2.06| 0.12|
| La Plata River at Colo./N.M. Border                                   | 0.41| 0.29| 1.32| 0.69|     |     |     | 1.53| 0.23|
| Control                                                               |     |     |     |     |     |     |     | 1.47| 1.04|
| Control + EDTA                                                        |     |     |     |     |     |     |     |     | 1.70|
| 3/8/78
| Animas River at Durango                                               | 0.26| 0.39| 1.21| 1.17| 0.18| 0.10| 1.65| 0.09| 0.92|
| Animas River at 32nd St. Bridge                                        | 0.12| 0.12| 1.25| 1.25| 0.14| 0.19| 1.65| 0.00| 0.66|
| La Plata River at Colo./N.M. Border                                   | 0.38| 0.09| 0.62| 0.76| 0.29| 0.29| 0.71| 0.07| 0.74|
| Control                                                               |     |     |     |     |     |     |     | 1.58|     |
| Control + EDTA                                                        |     |     |     |     |     |     |     |     | 1.50|
Table III-7.
Animas - La Plata Project
Maximum Specific Growth Rate, $\hat{u}_b$, dag-1 a

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a The maximum specific growth rate ($\hat{u}_b$) for an individual treatment is the largest specific growth rate ($u_b$) occurring at any time during incubation. The specific growth rate, $u_b$, is defined by:

$$u_b = \frac{\ln(X_2/X_1)}{t_2 - t_1}$$

where

$X_2$ = biomass concentration at end of selected time interval

$X_1$ = biomass concentration at beginning of selected time interval

$t_2 - t_1$ = elapsed time (in days) between selected determinations of biomass
Results and Interpretation:

Table III-8 indicates the nutrient limitation as a result of chemical analysis and as a result of algal bioassay for all sites on the Animas-La Plata Project. While nitrogen and phosphorus are most often the algal growth limiting nutrients, it should be recognized that other nutrients may be growth limiting. Theoretical productivity potential must be verified by actual algal assay analyses to determine: (1) the presence of growth limiting nutrients; (2) the presence of toxicants such as heavy metals and (3) if the chemical analyses for N and P is realistic.

1. Animas River at Durango - Figures 1-20

In September 1977 this river sample was phosphorus limited first and then nitrogen limited. The following bioassays in November, 1977; January 1978; March, 1978; and May, 1978 showed similar responses. Without the addition of nutrients, there was only limited response, if any at all, and the river seemed to be oligotrophic. However, the most important fact determined as a result of algal assay was a severe metal toxicity problem on the Animas River at Durango. Growth without addition of Ethylenedinitrite tetraacetic acid (EDTA) ranged from approximately 10 percent to 40 percent of normal depending on the heavy metal concentration during any particular sampling period. Organic chelation of heavy metals with EDTA created conditions more conducive to algal growth and as a result growth was 80 to 100 percent of normal as compared to an AAM control.

Based on a limited amount of heavy metal data available for the Animas River at Durango, it appears likely that zinc was the toxic
Table III-8.

Anímas - La Plata Project

Limiting Nutrients

<table>
<thead>
<tr>
<th>Sample</th>
<th>Limiting Nutrient(s)</th>
<th>Chemical Analysis</th>
<th>Bioassay</th>
</tr>
</thead>
</table>
| 9/8/77
Anímas River at Durango                    | Nitrogen                     |                   | Phosphorus |
| Anímas River at 32nd St. Bridge             | Nitrogen                     |                   | Phosphorus |
| 11/29/77
Anímas River at Durango                    | Phosphorus                   |                   | Nitrogen & Phosphorus |
| Anímas River at 32nd St. Bridge             | Phosphorus                   |                   | Nitrogen & Phosphorus |
| La Plata River at Colo./N.M. Border         | Phosphorus                   |                   | Phosphorus |
| 1/9/78
Anímas River at Durango                    | Phosphorus                   |                   | Phosphorus |
| Anímas River at 32nd St. Bridge             | Phosphorus                   |                   | Phosphorus |
| La Plata River at Colo./N.M. Border         | Phosphorus                   |                   | Phosphorus |
| 3/8/78
Anímas River at Durango                    | Phosphorus                   |                   | Phosphorus |
| Anímas River at 32nd St. Bridge             | Phosphorus                   |                   | Phosphorus |
| La Plata River at Colo./N.M. Border         | Phosphorus                   |                   | Phosphorus |
| 5/10/78
Anímas River at Durango                    | Phosphorus                   |                   | Nitrogen & Phosphorus |
| Anímas River at 32nd St. Bridge             | Phosphorus                   |                   | Nitrogen & Phosphorus |
| La Plata River at Colo./N.M. Border         | Phosphorus                   |                   | Nitrogen & Phosphorus |

1 Addition of phosphorus substantially increases the maximum specific growth rate; $\hat{\mu}_b$ (Table 7) indicating phosphorus limitation. However, due to the low level of both indigenous nitrogen and phosphorus growth is only minimal upon phosphorus addition (Table 5) as nitrogen becomes limiting as well.
metal in the river. Studies conducted by Joseph C. Green, et al. (1976) on algal growth in Long Lake, Washington have shown that zinc, cadmium or copper create toxic conditions for algae. Neither copper nor cadmium were at toxic concentrations during any of the five sampling dates. In fact both of these elements remained at a level below the detectable limit of the Varian Atomic Absorption Spectrophotometer used to measure the elements. On the other hand a definite correlation between zinc concentration and algal biomass was noted as represented by Figure 1-A. Greene, et al., found that zinc levels of 0.003 - 0.121 mg/l were toxic on Long Lake. The zinc levels on the Animas River at Durango were within this range and higher.

The zinc concentration remained high throughout the fall and winter months but the last assay in May, 1978 indicated a dilution effect on the zinc concentration probably due to the spring turnover. Biomass in Treatment H increased from an average of 11.5 mg/l V.S.S. to 34.7 mg/l V.S.S. Nevertheless, the zinc concentration was still high enough to limit increase in biomass upon N and P addition to less that 40 percent of normal.

As a result of the high zinc concentration in the Animas River at Durango, it seems unlikely that productivity will increase even upon addition of higher concentrations of nitrogen and phosphorus. The main concern appears to be the heavy metals concentration and consequently the possible toxic effect on aquatic organisms. Chapter IV provides a more thorough discussion of the trophic status on the Animas River.
ANIMAS RIVER AT DURANGO
SEPTEMBER 8 1977

+ = 1/1 RRM CONTROL.

OPTICAL DENSITY (750 NM. I CM.)

TIME (DAYS)
Figure 3.

Inamas River at Durango
September 8, 1977

- A = TRT A B E F
- C = TRT C
- D = TRT D 2
- G = TRT G
- + = 1/1 AAM + EDTA
- CONTROL

Time (Days)

Relative Fluorescence x 30
ANIMAS RIVER AT DURANGO
SEPTEMBER 8 1977

H = TRT H
I = TRT I
+ = I/I AAM CONTROL

Figure 4.

RELATIVE FLUORESCENCE X 30

TIME [DAYS]
ANIMAS RIVER AT DURANGO
NOVEMBER 29 1977

A = TRTS A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

Figure 5.
ANIMAS RIVER AT DURANGO
NOVEMBER 29 1977.

H = TRT H
I = TRT I
+ = 1/2 AAM CONTROL

OPTICAL DENSITY [750 NM. | CM.]

0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000

TIME [DAYS]

0.  2.  4.  6.  8.  10.  12.  14.  16.  18.

Figure 6.
ANIMAS RIVER AT DURANGO
NOVEMBER 29 1977

A = TRTS A B E F
C = TRT C
D = TRT D
G = TRT G
+= 1/2 AAM + EDTA
CONTROL

Figure 7.
ANIMAS RIVER AT DURANGO

H = TRT H
I = TRT I
+ = 1/2 RAM CONTROL

Figure 8.
ANIMAS RIVER AT DURANGO
JANUARY 9 1978

A = TRTS A & B
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

Figure 9.
ANIMAS RIVER AT DURANGO
JANUARY 9 1978

H = TRT H
L = TRT L
+ = 1/2 AAM CONTROL

Figure 10.
Figure 11.

ANIMAS RIVER AT DURANGO
JANUARY 9 1978

A = TRTS A B
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
ANIMAS RIVER AT DURANGO
JANUARY 9 1978

H = TRT H
I = TRT I
+ = 1/2 AAM CONTROL

Figure 12.
Figure 13.
Figure 15.

ANIMAS RIVER AT DURANGO
MARCH 9 1978

A = TRTS A B C E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

RELATIVE FLUORESCENCE X 30

TIME [DAYS]
ANIMAS RIVER AT DURANGO
MARCH 8, 1978

H = TRT H
I = TRT I
+ = 1/2 AAM CONTROL

RELATIVE FLUORESCENCE X 30

TIME [DAYS]

Figure 16.
ANIMAS RIVER AT DURANGO
MAY 10, 1972

A = TRT A
B = TRT B
+ = 0.75 FLAM CONTROL

Figure 18.
ANIMAS RIVER AT DURANGO
MAY 10, 1978

A = TRT A, B, C, D, E, F + EDTA
D = TRT D + EDTA
E = TRT E + EDTA

+ = 1/2 RAN CONTROL + EDTA

RELATIVE FLUORESCENCE X 30

1750.
1500.
1250.
1000.
750.
500.
250.

TIME (DAYS)
1. 2. 3. 4. 5. 6. 7. 8. 9.

Figure 19.
ANIMAS RIVER AT DURANGO
MAY 1-10 1976

\[ A = TAT A \]
\[ B = TAT B \]
\[ C = TAT C \]
\[ + = 1/2 ABM CONTROL \]

TIME [DAYS]
2. Animas River at 32nd Street Bridge - Figure 21-40

Results of the algal assays at the 32nd Street Bridge site suggested a situation similar to that found at the Durango site. The sample was limited by both nitrogen and phosphorus at each sampling date, with phosphorus being the most limiting. A heavy metal toxicity problem was also indicated. Due to a definite increase in algal growth upon treatment with EDTA as compared to no EDTA addition, heavy metals were definitely pinpointed as the cause of toxicity. However, no heavy metals data were available for this site upon which to base any assumptions concerning particular metals involved. The close proximity of the Animas River sites (only several miles apart) leads one to the conclusion that zinc played a role in toxicity at the 32nd Street Bridge site as well as at the Animas site. The degree of toxicity at this site didn't follow the same pattern observed at the Durango site, however. The toxicity level varied from month to month with no definite pattern. This fact coupled with no available heavy metals data makes it impossible to draw any valid assumptions concerning the cause of toxicity on the Animas River at 32nd Street Bridge.

The low indigenous concentration of nitrogen and phosphorus as determined by chemical analysis was verified by algal bioassay. This extremely low nutrient level along with the heavy metal toxicity point in the direction of an oligotrophic to mesotrophic condition at this site on the Animas River.
ANIMAS RIVER AT 32ND STREET BRIDGE
SEPTEMBER 8 1977

A = TRTS A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/1 AAM + EDTA
CONTROL

**Figure 21.**
ANIMAS RIVER AT 32ND STREET BRIDGE
SEPTEMBER 8, 1977

OPTICAL DENSITY (750 NM, 1 CM)

I = +1/1 AAM CONTROL

TIME (DAYS)
ANIMAS RIVER AT 32ND STREET BRIDGE
SEPTEMBER 8 1977

A = TRTS A B E F
C = TRT C
D = TRT D 2
G = TRT G
+ = 1/1 ARM + EDTA
CONTROL

RELATIVE FLUORESCENCE X 30

250.

2000.

1500.

1000.

750.

500.

250.

0.

1.

2.

3.

4.

5.

6.

7.

8.

9.

TIME (DAYS)

Figure 23.
ANIMAS RIVER AT 32ND STREET BRIDGE
SEPTEMBER 8 1977

H = TRT H
I = TRT I
+ = I/I AAM CONTROL

Figure 24
ANIMAS RIVER AT 32ND STREET BRIDGE
NOVEMBER 29 1977

A = TRTS A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 RAB + EDTA
CONTROL

OPTICAL DENSITY [750 NM. 1 CM.]

0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000

TIME [DAYS]

Figure 25.
ANIMAS RIVER AT 32ND STREET BRIDGE
NOVEMBER 29 1977.

H = TRT H
I = TRT I
+ = 1/2 AAM CONTROL

Figure 26.
ANIMAS RIVER AT 32ND STREET BRIDGE
NOVEMBER 29 1977

A = TRTS A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 RRM + EDTA
CONTROL

Figure 27.
ANIMAS RIVER AT 32ND STREET BRIDGE

H = TRT H
I = TRT I
+ = 1/2 AAM CONTROL

Figure 28.
ANIMAS RIVER AT 32ND STREET BRIDGE
JANUARY 9 1978

A = TRTS A B
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 RMM + EDTA
CONTROL

OPTICAL DENSITY [(750 NM. / CM.)

TIME [DAYS]

Figure 29.
ANIMAS RIVER AT 32ND STREET BRIDGE.
JANUARY 9 1978

H = TRT H
I = TRT I
+ = 1/2 RAR CONTROL

OPTICAL DENSITY [750 NM, 1 CM]

0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000

0
2
4
6
8
10
12
14
16
18

TIME [DAYS]

Figure 30.
ANIMAS RIVER AT 32ND STREET BRIDGE
JANUARY 9 1978

A = TRTS A & B
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

Figure 31.
ANIMAS RIVER AT 32ND STREET BRIDGE
JANUARY 9 1978
H = TRT  H
I = TRT  I
+ = 1/2 ARM CONTROL

0
RELATIVE FLUORESCENCE X 30

TIME (DAYS)
250
500
750
1000
1250
1500
1750
2000
2250
0.0
1.5
3.0
4.5
6.0
7.5
9.0
10.5
1.20
1.35

Figure 32.
ANIMAS RIVER AT 32ND STREET BRIDGE
MARCH 8 1978.

A = TRTS A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

Figure 33.
ANIMAS RIVER AT 32ND STREET BRIDGE.
MARCH 8 1978

H = TRT H
I = TRT I
+ = 1/2 ARM CONTROL

Figure 34.
Figure 35.
ANIMAS RIVER AT 32ND STREET BRIDGE
MARCH 8 1978

H = TRT H
I = TRT I
+ = 1/2 ARM CONTROL

RELATIVE FLUORESCENCE X 30

TIME (DAYS)

Figure 36.
ANIMAS RIVER AT 32ND ST. BRIDGE
MAY 10, 1978

A = TRTS. A, B, E, F + EDTA
C = TRT C + EDTA
D = TRT D + EDTA
G = TRT G + EDTA
+ = 1/2 RRM CONTROL + EDTA

Figure 37.
ANIMAS RIVER AT 32ND ST. BRIDGE
MAY 10 1978

A = TRT A
G = TRT G
+ = 1/2 AAM CONTROL.

Figure 38.
Figure 40.
Chemical analysis as well as bioassay indicated phosphorus limitation first and then nitrogen limitation during November, January and March. In May, 1978 the sample was simultaneously limited by nitrogen and phosphorus. The May bioassay also showed a eutrophic response in the sample with no treatment as opposed to an oligotrophic response in all previous bioassays. The results of chemical analysis (Table III-1) indicated a greater concentration of phosphorus in May 1978 as compared to the previous months. The higher level of phosphorus created a near optimum M/P ratio of 22 and the result was greater productivity during the bioassay growth.

It should be noted that response upon addition of complete AAM during November, January, and March was significantly less than the AAM control. This was found to be the case regardless of whether EDTA was added or not. Often when this type of low algal response is observed it can be attributed to the hardness of the water and most specifically to the calcium concentration. Euster (1958) showed that photosynthetic activity caused removal of CO$_2$ resulting in higher pH. At the higher pH, the hardness precipitates, largely as CaCO$_3$ and/or coprecipitates Ca$_x$(PO$_4$)$_y$ compounds and heavy metals. Therefore, as the pH rises above pH 8.8, the CaCO$_3$ precipitate is formed removing CO$_2$ and other nutrients from the sample thus inhibiting algal growth and ultimately altering the results.

The calcium concentration in the La Plata River at State Line during November, January, March and May seemed to correlate well with the
observed algal response. In March, 1978 when algal response was the lowest, the calcium concentration (203 mg/l) was at the highest level observed during the entire monitoring period of 16 months. The calcium concentration in May, 1978 was at the lowest observed level (49 mg/l) and consequently the algal productivity was higher than during any other bioassay. The other algal bioassays at this site reflect the same trend. However, four bioassays do not provide a statistically sound basis from which to draw definite conclusions concerning calcium concentration as it relates to algal productivity in the La Plata River. Without further data, it may be speculated that the high calcium concentration created the lower than normal algal response.
LA PLATA RIVER AT COLO./N.M. BORDER
NOVEMBER 29 1977

A = TRT A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 RAM + EDTA
CONTROL

OPTICAL DENSITY [750 NM, 1 CM]

Figure 41.
LA PLATA RIVER AT COLO./N.M. BORDER
NOVEMBER 29 1977.

H = TRT H
I = TRT I
+ = 1/2 AAM CONTROL

Figure 42.
LA PLATA RIVER AT COLO./N.M. BORDER

H = TRT H
I = TRT I
+ = 1/2 RAM CONTROL

Figure 44.

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
LA PLATA RIVER AT COLO./N.M. BORDER
NOVEMBER 29 1977

A = TRTS A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

RELATIVE FLUORESCENCE X 30

2250.
2000.
1750.
1500.
1250.
1000.
750.
500.
250.

3.0 1.2 2.4 3.6 4.8 6.0 7.2 8.4 9.6 10.8
TIME (DAYS)

Figure 43.
LA PLATA RIVER AT COLO./N.M. BORDER
JANUARY 9 1978

OPTICAL DENSITY (750 NM, I CM)]

Figure 45.
Figure 46.

Optical Density (750 nm, 1 cm)
LA PLATA RIVER AT COLORADO. BORDER
JANUARY 9 1978

A = TRT A B
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL

RELATIVE FLUORESCENCE X 30

2500.
2000.
1750.
1500.
1250.
1000.
750.
500.
250.

0.0 1.5 3.0 4.5 6.0 7.5 9.0 10.5 12.0 13.5
TIME (DAYS)

Figure 47.
LA PLATA RIVER AT COLO./N.M. BORDER
JANUARY 3 1978

H = TRT. H
I = TRT I
+ = 1/2 AAM CONTROL

Figure 48.

TIME (DAYS)
Figure 49.

LA PLATA RIVER AT COLO./N.M. BORDER
MARCH 8 1978

A = TRT S A B E F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL
LA PLATA RIVER AT COLO./N.M. BORDER
MARCH 8 1978

H = TRT H
I = TRT I
+ = 1/2 FAM CONTROL

OPTICAL DENSITY [750 NM, 1 CM]

TIME [DAYS] 0

Figure 50.
LA PLATA RIVER AT COLO./N.M. BORDER
MARCH 8 1978

A = TRT S A B E F
C = TRT C
D = TRT D
G = TRT G

+ = 1/2 AAM + EDTA
CONTROL.

RELATIVE FLUORESCENCE X 30

2250.
2000.
1750.
1500.
1250.
1000.
750.
500.
250.

TIME [DAYS]

Figure 51.
LA PLATA RIVER AT COLO./N.M. BORDER
MARCH 8 1978.

H = TRT H
I = TRT I
+ = 1/2 AAM CONTROL

Figure 52.

RELATIVE FLUORESCENCE X 30
2500.
2000.
1750.
1500.
1250.
1000.
750.
500.
250.
0.
0.
2.
1.
3.
4.
5.
6.
7.
8.
9.

TIME (DAYS)
LA PLATA RIVER AT COLO./N.M. BORDER
MAY 10, 1978

A = TRTS A, B, C, E, F
D = TRT D
G = TRT G
+ = 1/2 RAM CONTROL

Figure 53.
Figure 54.

**LA PLATA RIVER AT COLO./N.M. BORDER**

**MAY 10, 1978**

- **A** = TRTS A, B, C, E, F
- **D** = TRT D
- **G** = TRT G
- **+** = 1/2 AAM CONTROL

**RELATIVE FLUORESCENCE X 30**

**TIME (DAYS)**
Conclusions

1. Animas River at Durango
   a. A large concentration of heavy metals (possibly zinc) resulted in an infertile sample.
   b. The sample was limited by both phosphorus and nitrogen, after heavy metals were made unavailable using EDTA, indicating infertility even when toxicity was removed.
   c. There was a good correlation between chemical analysis and algal bioassay.
   d. This sample represents a non-productive body of water with oligotrophic to mesotrophic tendencies in the future.

2. Animas River at 32nd St. Bridge
   a. Heavy metal toxicity was indicated at this site.
   b. The sample was limited by both nitrogen and phosphorus during each sampling period with phosphorus being the most limiting.
   c. Algal bioassay confirmed the chemical analysis.
   d. An oligotrophic to mesotrophic condition can be expected at this site.

3. La Plata River at Colorado/New Mexico Border
   a. Hardness, calcium hardness specifically, seemed the most likely cause of lower than normal productivity.
   b. Phosphorus was the limiting nutrient in all bioassay but nitrogen became limiting as well.
   c. Chemical analyses and bioassay correlated well.
   d. The sample was classified as mesotrophic even though productivity increased slightly during the spring. The increased productivity in May, 1978 was not substantial enough to classify the sample as becoming eutrophic.
REFERENCES


