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ALGAL BIOASSAY STUDY
for the
DOLORES PROJECT
DOMINGUEZ PROJECT
SAN MIGUEL PROJECT
WEST DIVIDE 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 DOLORES PROJECT,
DOMINGUEZ PROJECT, SAN MIGUEL PROJECT,
WEST DIVIDE PROJECT

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Leslie G. Terry
V. Dean Adams

This report was completed for the United States Bureau of Reclamation as a part of Contract No. 7-07-40-S0329 (Chemical and Biological Analysis of Colorado Water Samples).

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

June 1979
ACKNOWLEDGMENTS

Special thanks is given to Donald B. Porcella, Alberta Seierstad, Larry Baker, and Paula Bramble. Appreciation is also extended to the Utah Water Research Laboratory for use of their facilities.
I. Sample Pretreatment

Sample pretreatment was conducted in a similar manner for each of five sampling dates (September 8, 1977; November 29, 1977; January 9, 1978; March 8, 1978 and May 10, 1978) and for each of the samples from the four project sites. Immediately upon arrival three liters of each sample were filter sterilized using 0.45 μ millipore membrane filters. Filtering removes native algae and bacteria from the test water and enables the use of uni-algal 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 inorganic nitrogen (Tables 1 - 4). Also listed are total inorganic nitrogen/ortho phosphorus ratios as determined by chemical analysis.

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 measureable response of living organisms to environmental variables including determining whether or not nutrients are biologically available.

II. Experimental 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.
Table 1.
Dolores 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 (NH₄-N) (µg/l)</th>
<th>Nitrate+Nitrite (NO₃+NO₂)⁻-N (µg/l)</th>
<th>Nitrogen/Phosphorus* (NH₃+NO₃⁺+NO₂⁻-N)/PO₄-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/8/1977</td>
<td>Dolores River at Dolores</td>
<td>3.</td>
<td>13.</td>
<td>60.</td>
<td>35.</td>
<td>32.</td>
</tr>
<tr>
<td></td>
<td>Dolores River below Rico Tailings</td>
<td>1.</td>
<td>8.</td>
<td>64.</td>
<td>52.</td>
<td>116.</td>
</tr>
<tr>
<td></td>
<td>Dolores River above Rico Tailings</td>
<td>1.</td>
<td>7.</td>
<td>40.</td>
<td>112.</td>
<td>152.</td>
</tr>
<tr>
<td></td>
<td>Dolores River below West Dolores River</td>
<td>4.</td>
<td>7.</td>
<td>46.</td>
<td>41.</td>
<td>22.</td>
</tr>
<tr>
<td>5/10/1978</td>
<td>Dolores River at Dolores</td>
<td>&lt;1.</td>
<td>4.</td>
<td>17.</td>
<td>80.</td>
<td>&gt;97.</td>
</tr>
</tbody>
</table>

*A nitrogen/phosphorus ratio of <15 indicates nitrogen limitation while an N/P ratio of >15 indicates phosphorus limitation.
<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-N+NO₂-N) (µg/l)</th>
<th>Nitrogen/Phosphorus* (NH₃-N+NO₃-N+NO₂-N)/PO₄-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/10/1978</td>
<td>Gunnison River near Grand Junction</td>
<td>17.</td>
<td>32.</td>
<td>54.</td>
<td>900.</td>
<td>56.</td>
</tr>
</tbody>
</table>

*A nitrogen/phosphorus ratio of <15 indicates nitrogen limitation while an N/P ratio of >15 indicates phosphorus limitation.*
| Date       | Location                        | Orthophosphate (PO₄-P) (µg/l) | Total Soluble Phosphorus (µg/l) | Ammonia (NH₄-N) (µg/l) | Nitrate-Nitrite (NO₃-N+NO₂-N) (µg/l) | Nitrogen/Phosphorus Ratio *
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>9/8/1977</td>
<td>San Miguel River near Placerville</td>
<td>6.</td>
<td>11.</td>
<td>42.</td>
<td>164.</td>
<td>34.</td>
</tr>
<tr>
<td></td>
<td>San Miguel River near Sawpit</td>
<td>13.</td>
<td>23.</td>
<td>61.</td>
<td>245.</td>
<td>24.</td>
</tr>
<tr>
<td></td>
<td>Leopard Creek</td>
<td>1.</td>
<td>7.</td>
<td>64.</td>
<td>31.</td>
<td>95.</td>
</tr>
<tr>
<td></td>
<td>San Miguel River near Sawpit</td>
<td>12.</td>
<td>34.</td>
<td>54.</td>
<td>390.</td>
<td>37.</td>
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<tr>
<td></td>
<td>Leopard Creek</td>
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<td>80.</td>
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<tr>
<td></td>
<td>San Miguel River near Sawpit</td>
<td>12.</td>
<td>23.</td>
<td>90.</td>
<td>320.</td>
<td>34.</td>
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<tr>
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<td>3.</td>
<td>14.</td>
<td>2.</td>
<td>100.</td>
<td>55.</td>
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<td></td>
<td>San Miguel River near Sawpit</td>
<td>22.</td>
<td>41.</td>
<td>21.</td>
<td>339.</td>
<td>16.</td>
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<td></td>
<td>Leopard Creek</td>
<td>2.</td>
<td>9.</td>
<td>27.</td>
<td>73.</td>
<td>50.</td>
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<tr>
<td></td>
<td>San Miguel River near Sawpit</td>
<td>2.</td>
<td>3.</td>
<td>98.</td>
<td>150.</td>
<td>124.</td>
</tr>
</tbody>
</table>

*A nitrogen/phosphorus ratio of <15 indicates nitrogen limitation while an N/P ratio of >15 indicates phosphorus limitation.
Table 4.
West Divide 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>Nitrite+Nitrate (NO₂⁻+NO₃⁻) (µg/l)</th>
<th>Nitrogen/Phosphorus (NH₃-N+NO₂⁻+NO₃⁻)/PO₄-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/29/1977</td>
<td>Colorado River at Newcastle</td>
<td>2.</td>
<td>28.</td>
<td>5.</td>
<td>3.</td>
<td>4.</td>
</tr>
<tr>
<td></td>
<td>(upstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colorado River at Newcastle</td>
<td>2.</td>
<td>40.</td>
<td>4.</td>
<td>8.</td>
<td>6.</td>
</tr>
<tr>
<td></td>
<td>(downstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/9/1978</td>
<td>Colorado River at Newcastle</td>
<td>17.</td>
<td>28.</td>
<td>70.</td>
<td>310.</td>
<td>22.</td>
</tr>
<tr>
<td></td>
<td>(upstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colorado River at Newcastle</td>
<td>17.</td>
<td>37.</td>
<td>49.</td>
<td>310.</td>
<td>21.</td>
</tr>
<tr>
<td></td>
<td>(downstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(upstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colorado River at Newcastle</td>
<td>17.</td>
<td>96.</td>
<td>43.</td>
<td>175.</td>
<td>13.</td>
</tr>
<tr>
<td></td>
<td>(downstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(upstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Colorado River at Newcastle</td>
<td>12.</td>
<td>15.</td>
<td>55.</td>
<td>240.</td>
<td>25.</td>
</tr>
<tr>
<td></td>
<td>(downstream)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A nitrogen/phosphorus ratio of <15 indicates nitrogen limitation while an N/P ratio of >15 indicates phosphorus limitation.*
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, 1:2 hydrochloric acid rinses, deionized water rinses and finally ultra pure deionized water rinses. Following washing, all glassware was autoclaved using aluminum foil closures at 121°C for 15 minutes.

Samples from each of the projects received the treatments listed on Tables 5 - 8 for each of the five sampling dates. Each treatment was set up in triplicate. The sample blanks (treatments A and H) were included to provide the basis for comparison of the other treatments and to provide a measure of general fertility of the sample. The control treatments were included to provide an estimate of theoretical maximum cell growth and an index for comparing growth levels of the test waters.

Table 9 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 controls (with the exception of 9/8/77 controls which contained full strength AAM for all constituents) contained one half AAM levels of nitrogen and phosphorus whereas all other constituents 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 toxic metal ions biologically inactive. Metal toxicity was detected in earlier bioassays from the heavily mined western Colorado area, therefore as a precautionary measure for the detection of metal toxicity the EDTA addition was made. Treatments H and I (with EDTA) were included to confirm any metal toxicity. Increased growth in EDTA spiked flasks in
Table 5.
Dolores Project
Treatment Constituents

9/8/77
Dolores River below Rico Tailings
Dolores River above Rico Tailings
Dolores River at Dolores
Dolores River below West Dolores River
A. Sample + 1 mg/l EDTA
B. Sample + 1 mg/l EDTA + 2.1 mg/l Nitrogen (N)
C. Sample + 1 mg/l EDTA + 0.093 mg/l Phosphorus (P)
D. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P
E. Sample + 1 mg/l EDTA + trace element (AAM levels)
F. Sample + 1 mg/l EDTA + 15.0 mg/l NaHCO₃
G. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄
H. Sample
I. Sample + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄
Control: Distilled water + 4.2 mg/l N + 0.186 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄
Control + EDTA: Distilled water + 1 mg/l EDTA + same as control above.

11/29/77
Dolores River at Dolores
Sample treatments same as 9/8/77
Control: Distilled water + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄
Control + EDTA: Distilled water + 1 mg/l EDTA + same as control above.

1/9/78
Dolores River at Dolores
Sample treatments same as 11/29/77 except treatments E and F eliminated due to lack of sample
Controls same as 11/29/77

3/8/78
Dolores River at Dolores
Sample treatments and controls same as 11/29/77

5/10/78
Dolores River at Dolores
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 level)
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
Table 6.
Dominquez Project
Treatment Constituents

11/29/77
Gunnison River at Grand Junction
A. Sample + 1 mg/l EDTA
B. Sample + 1 mg/l EDTA + 2.1 mg/l Nitrogen (N)
C. Sample + 1 mg/l EDTA + 0.093 mg/l Phosphorus (P)
D. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P
E. Sample + 1 mg/l EDTA + trace elements (AAM level)
F. Sample + 1 mg/l EDTA + 15.0 mg/l NaHCO₃
G. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄
H. Sample
I. Sample + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄

Control: Distilled water + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂, and MgSO₄
Control + EDTA: Distilled water + 1 mg/l EDTA + same as control above.

1/9/78
Gunnison River at Grand Junction
Sample treatments same as 11/29/77 except treatments E and F eliminated due to lack of sample.
Controls same as 11/29/77.

3/8/78
Gunnison River at Grand Junction
Sample treatments and controls same as 11/29/77

5/10/79
Gunnison River at Grand Junction
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
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
<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Sample Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/8/77</td>
<td>San Miguel River near Placerville</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 + 0.093 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: 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: trace elements, NaHCO₃, CaCl₂, and MgSO₄</td>
</tr>
<tr>
<td></td>
<td>Control: Distilled water + 4.2 mg/l N + 0.186 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂, and MgSO₄</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control + EDTA: Same as control above + 1 mg/l EDTA</td>
<td></td>
</tr>
<tr>
<td>11/29/77</td>
<td>San Miguel River near Placerville</td>
<td>Sample treatments same as 9/8/77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control: Distilled water + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control + EDTA: Same as above + 1 mg/l EDTA</td>
</tr>
<tr>
<td>1/9/78</td>
<td>San Miguel River near Placerville</td>
<td>Sample treatments same as 11/29/77 except treatments E and F were eliminated due to lack of sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls same as 11/29/77</td>
</tr>
<tr>
<td>3/8/78</td>
<td>San Miguel River near Placerville</td>
<td>Sample treatments and controls same as 11/29/77</td>
</tr>
<tr>
<td>5/10/78</td>
<td>San Miguel River near Placerville</td>
<td></td>
</tr>
</tbody>
</table>
Table 7. Continued.
San Miguel Project
Treatment Constituents

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sample</td>
</tr>
<tr>
<td>B</td>
<td>Sample + 2.1 mg/l N</td>
</tr>
<tr>
<td>C</td>
<td>Sample + 0.093 mg/l P</td>
</tr>
<tr>
<td>D</td>
<td>Sample + 2.1 mg/l N + 0.093 mg/l P</td>
</tr>
<tr>
<td>E</td>
<td>Sample + trace elements (AAM levels)</td>
</tr>
<tr>
<td>F</td>
<td>Sample + 15.0 mg/l NaHCO₃</td>
</tr>
<tr>
<td>G</td>
<td>Sample + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td></td>
<td>Control same as 11/29/77</td>
</tr>
</tbody>
</table>

San Miguel River Sawpit

After several days of incubation very little growth resulted. Metal toxicity was suspected therefore the following treatments were added.

<table>
<thead>
<tr>
<th></th>
<th>Sample + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄ (this treatment was repeated as a check on the fertility of the sample).</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄</td>
</tr>
<tr>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>
Table 8.
West Divide Project
Treatment Constituents

11/29/79
Colorado River at Newcastle (upstream)
Colorado River at Newcastle (downstream)
A. Sample + 1 mg/l EDTA
B. Sample + 1 mg/l EDTA + 2.1 mg/l Nitrogen (N)
C. Sample + 1 mg/l EDTA + 0.093 mg/l Phosphorus (P)
D. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P
E. Sample + 1 mg/l EDTA + trace elements
F. Sample + 1 mg/l EDTA + 15.0 mg/l NaHCO₃
G. Sample + 1 mg/l EDTA + 2.1 mg/l N + 0.093 mg/l P + AAM
   levels of: trace elements, NaHCO₃, CaCl₂, and MgSO₄
H. Sample
I. Sample + 2.1 mg/l N + 0.093 mg/l P + AAM levels of: trace elements, NaHCO₃, CaCl₂ and MgSO₄.
Control: Distilled water + 2.1 mg/l N + 0.093 mg/l P -- AAM levels
   of trace elements, NaHCO₃, CaCl₂ and MgSO₄.
Control + EDTA: Same as control above + 1 mg/l EDTA.

1/9/78
Colorado River at Newcastle (upstream)
Colorado River at Newcastle (downstream)
Sample treatments same as 11/29/77 except treatments E and F
   eliminated due to lack of sample.
Controls same as 11/29/77

3/8/78
Colorado River at Newcastle (upstream)
Colorado River at Newcastle (downstream)
Sample treatments and controls same as 11/29/77

5/10/78
Colorado River at Newcastle (upstream)
Colorado River at Newcastle (downstream)
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
F. Sample + 15.0 mg/l NaHCO₃
G. Sample + 2.1 mg/l + 0.093 mg/l + AAM levels of: trace elements, NaHCO₃, CaCl₂, and MgSO₄
Control: Same as 11/29/77
Table 9. Algal Assay medium (AAM)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in NAAM</th>
<th>Element</th>
<th>mg/l</th>
<th>Element</th>
<th>µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt; NaNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>25.500</td>
<td>N</td>
<td>4.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt; MgCl&lt;sub&gt;2&lt;/sub&gt; 6H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>12.171</td>
<td>Mg</td>
<td>2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgSO&lt;sub&gt;4&lt;/sub&gt; 7H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>14.700</td>
<td>Ca</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
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Protocol for Nutrient Spiking

A<sub>1</sub> Nitrogen
B Phosphorus
A<sub>1</sub> + B N + P
C + D Trace Elements (T. E.)
ALL NAAM

comparison to yields in untreated flasks can be directly attributed 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°C ± 2°C) with "cool white" fluorescent lighting providing illumination of 400 ft-C (4304 lux) ± 10 percent.

The algal bioassays were monitored by determining the optical density (OD Bausch and Lomb Spectrophotometer 70 at 750 nm, 1 cm path length) and 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 sensitivity. 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 chlorophyll a is increasing so is biomass and vice versa. Maximum values for optical density are listed on Tables 10 - 13.

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 (VSS), can be calculated directly from OD. The relationship used to convert OD to VSS in Tables 14-17 is:

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

Because of the difficulty of measuring biomass in low density cultures, relative fluorescence of *in vivo* chlorophyll a was used to estimate biomass
Table 10.
Dolores Project
Maximum Amount of Growth Observed As Optical Density; 750 mm., 1 cm.

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Table 11.
Dominquez Project
Maximum Amount of Growth Observed as Optical Density; 750 mm., 1 cm.

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Table 12.
San Miguel Project
Maximum Amount of Growth Observed as Optical Density; 750 mm., 1 cm.

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Table 12. Continued.
San Miguel Project
Maximum Amount of Growth Observed as Optical Density; 750 mm., 1 cm.

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Page 2 of 2
### Table 13.
**West Divide Project**
**Maximum Amount Of Growth Observed As Optical Density; 750 mm., 1 cm.**

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<th>B</th>
<th>C</th>
<th>D</th>
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Dolores Project
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a VSS = Volatile Suspended Solids
VSS, mg/1 = 350 (Optical Density) + 3.5 (Porcella, et al., 1973)
Table 15.
Dominquez 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 16.
San Miguel Project
Maximum Amount of Growth Observed as mg/l VSS.\textsuperscript{a}

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Table 16. Continued.
San Miguel Project
Maximum Amount of Growth Observed as mg/l VSS.\textsuperscript{a}

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\textsuperscript{a}VSS = Volatile Suspended Solids
VSS, mg/l = 350 (Optical Density) + 3.5 (Porcella, et al., 1973)
Table 17.
West Divide Project
Maximum Amount of Growth Observed as mg/1 VSS.\textsuperscript{a}

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\textsuperscript{a}VSS = Volatile Suspended Solids
VSS,mg/1 = 350 (Optical Density) + 3.5 (Porcella, et al., 1973)
in the early phases of the bioassay. Maximum values for relative fluorescence are listed on Tables 18 - 21. Calculations of average maximum specific growth rate batch \( \mu_b \) were made using relative fluorescence. The maximum specific growth occurs during the logarithmic phase of growth, usually between day 0 and day 5 and is useful in determining a sample response to each treatment. Maximum specific growth rates are calculated in Tables 22 - 25.

III. Results and Interpretation

Tables 26 - 29 outline the nutrient limitation as a result of chemical analysis and as a result of algal bioassay for all sites on all projects. While nitrogen and phosphorus are most often the algal growth limiting nutrient, it should be recognized that other nutrients may be growth limiting as well. 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 are realistic.

A. Dolores Project

1. Dolores River at Dolores (Figures 1 - 18)

Each bioassay at this sampling site showed similar net results. Indigenous nutrient levels remained consistently low with the amount of total inorganic nitrogen progressively increasing during the winter months reaching a maximum concentration of 326 µg/l just before spring runoff then rapidly declining to 97 µg/l when runoff occurred. However, at no time during the year did the nitrogen or phosphorus concentrations in the sample reach a level that resulted in a productive untreated sample. Increased biomass was observed only when both nitrogen and phosphorus were added to
Table 18.
Dolores Project
Maximum Amount of Growth Observed as Relative Fluorescence, RF x 30

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Table 19.
Dominquez Project
Maximum Amount of Growth Observed as Relative Fluorescence, RF x 30

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Table 20. Continued.
San Miguel Project
Maximum Amount of Growth Observed As Relative Fluorescence, RF x 30

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Table 21.
West Divide Project
Maximum Amount of Growth Observed As Relative Fluorescence, RF x 30

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Table 22.
Dolores Project
Maximum Specific Growth Rates; $\mu_b$, days$^{-1}$

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<th>B</th>
<th>C</th>
<th>D</th>
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<td>0.00</td>
<td>0.22</td>
<td>1.26</td>
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<td>1.81</td>
<td>0.00</td>
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The maximum specific growth rate ($\mu_b$) for an individual treatment is the largest specific growth rate ($\mu_b$) occurring at any time during incubation. The specific growth rate, $\mu_b$, is defined by:

$\mu_b = \frac{\ln(X_2/X_1)}{t_2 - t_1}$ days$^{-1}$

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
Table 23. Dominquez Project
Maximum Specific Growth Rate, $\hat{\mu}_b$, days $^{-1}$

<table>
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<tr>
<th>Sample</th>
<th>Treatment</th>
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<th>B</th>
<th>C</th>
<th>D</th>
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The maximum specific growth rate ($\hat{\mu}_b$) for an individual treatment is the largest specific growth rate ($\mu_b$) occurring at any time during incubation. The specific growth rate, $\mu_b$, is defined by:

$$\mu_b = \frac{\ln(X_2/X_1)}{t_2 - t_1} \text{ days}^{-1}$$

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
<table>
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<th>Sample</th>
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<td><strong>1/9/78</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>San Miguel River near Placerville</td>
<td>1.25</td>
<td>0.86</td>
<td>1.95</td>
<td>1.45</td>
<td></td>
<td></td>
<td>2.24</td>
<td>0.70</td>
<td>2.41</td>
</tr>
<tr>
<td>San Miguel River near Sawpit</td>
<td>0.43</td>
<td>0.88</td>
<td>1.30</td>
<td>1.27</td>
<td></td>
<td></td>
<td>2.37</td>
<td>0.29</td>
<td>1.94</td>
</tr>
<tr>
<td>Leopard Creek</td>
<td>0.15</td>
<td>0.31</td>
<td>0.42</td>
<td>1.50</td>
<td></td>
<td></td>
<td>1.79</td>
<td>0.22</td>
<td>1.94</td>
</tr>
<tr>
<td><strong>3/8/78</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>San Miguel River near Placerville</td>
<td>0.29</td>
<td>0.21</td>
<td>0.41</td>
<td>1.47</td>
<td>0.30</td>
<td>0.38</td>
<td>1.75</td>
<td>0.46</td>
<td>1.71</td>
</tr>
<tr>
<td>San Miguel River near Sawpit</td>
<td>0.91</td>
<td>1.05</td>
<td>1.24</td>
<td>1.57</td>
<td>0.99</td>
<td>1.02</td>
<td>1.79</td>
<td>0.94</td>
<td>1.70</td>
</tr>
<tr>
<td>Leopard Creek</td>
<td>0.34</td>
<td>0.59</td>
<td>0.69</td>
<td>1.41</td>
<td>0.59</td>
<td>0.20</td>
<td>1.64</td>
<td>0.31</td>
<td>1.63</td>
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<tr>
<td><strong>5/10/78</strong></td>
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<tr>
<td>San Miguel River near Placerville</td>
<td>0.07</td>
<td>0.12</td>
<td>0.64</td>
<td>0.74</td>
<td>0.35</td>
<td>0.13</td>
<td>1.66</td>
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<tr>
<td>San Miguel River near Sawpit</td>
<td>0.18</td>
<td>0.13</td>
<td>0.31</td>
<td>0.46</td>
<td>0.25</td>
<td>0.12</td>
<td>0.81</td>
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<tr>
<td>Leopard Creek</td>
<td>0.50</td>
<td>0.56</td>
<td>0.44</td>
<td>1.52</td>
<td>0.49</td>
<td>0.60</td>
<td>1.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

$$
\hat{\mu}_b = \frac{\ln(X_2/X_1)}{t_2 - t_1} \text{ days}^{-1}
$$

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
Table 25.
West Divide Project
Maximum Specific Growth Rate; $\hat{\mu}_b$, days $^{-1}$

<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>
</tr>
</thead>
<tbody>
<tr>
<td><strong>11/29/77</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River at Newcastle (Upstream)</td>
<td>0.25</td>
<td>0.34</td>
<td>0.33</td>
<td>2.52</td>
<td>0.17</td>
<td>0.31</td>
<td>2.08</td>
<td>0.17</td>
<td>2.54</td>
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<tr>
<td>Colorado River at Newcastle (Downstream)</td>
<td>0.32</td>
<td>0.34</td>
<td>0.14</td>
<td>2.64</td>
<td>0.13</td>
<td>0.17</td>
<td>1.90</td>
<td>0.10</td>
<td>2.40</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River at Newcastle (Upstream)</td>
<td>0.31</td>
<td>0.55</td>
<td>1.40</td>
<td>1.54</td>
<td></td>
<td></td>
<td>1.87</td>
<td>0.61</td>
<td>1.96</td>
</tr>
<tr>
<td>Colorado River at Newcastle (Downstream)</td>
<td>0.88</td>
<td>0.63</td>
<td>0.64</td>
<td>1.29</td>
<td></td>
<td></td>
<td>2.24</td>
<td>0.34</td>
<td>1.91</td>
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<tr>
<td>Colorado River at Newcastle (Upstream)</td>
<td>0.62</td>
<td>0.63</td>
<td>0.84</td>
<td>1.74</td>
<td>0.80</td>
<td>0.78</td>
<td>1.68</td>
<td>0.26</td>
<td>1.58</td>
</tr>
<tr>
<td>Colorado River at Newcastle (Downstream)</td>
<td>0.69</td>
<td>0.29</td>
<td>0.77</td>
<td>1.60</td>
<td>0.72</td>
<td>0.80</td>
<td>1.61</td>
<td>0.80</td>
<td>1.68</td>
</tr>
<tr>
<td><strong>5/10/78</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River at Newcastle (Upstream)</td>
<td>0.80</td>
<td>0.67</td>
<td>1.24</td>
<td>1.58</td>
<td>0.78</td>
<td>0.61</td>
<td>1.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River at Newcastle (Downstream)</td>
<td>0.73</td>
<td>0.71</td>
<td>1.07</td>
<td>1.65</td>
<td>0.61</td>
<td>0.57</td>
<td>1.61</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

$$\mu_b = \frac{\ln(X_2/X_1)}{t_2 - t_1} \text{ days}^{-1}$$

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
Table 26.
Dolores Project
Limiting Nutrients

<table>
<thead>
<tr>
<th>Sample</th>
<th>Limiting Nutrient(s)</th>
<th>Chemical Analysis</th>
<th>Bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/8/77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolores River Below Rico Tailings</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dolores River Above Rico Tailings</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dolores River at Dolores</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dolores River Below West Dolores River</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>11/29/77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolores River at Dolores</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen</td>
<td></td>
</tr>
<tr>
<td>1/9/78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolores River at Dolores</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen</td>
<td></td>
</tr>
<tr>
<td>3/8/78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolores River at Dolores</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen</td>
<td></td>
</tr>
<tr>
<td>5/10/78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dolores River at Dolores</td>
<td>Phosphorus</td>
<td>Phosphorus &amp; Nitrogen</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Addition of phosphorus substantially increased the maximum specific growth rate, \( \mu_p \), indicating phosphorus limitation. However, due to the low level of both indigenous nitrogen and phosphorus growth was only minimal upon phosphorus addition as nitrogen became limiting as well.
DOLORES RIVER AT DOLORES.
SEPTEMBER 8 1977

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

0.0 1.6 3.2 4.8 6.4 8.0 9.6 11.2 12.8 14.4
TIME [DAYS]

Figure 1.
DOLORES RIVER AT DOLORES
SEPTEMBER 8 1977

H = TAT H.
I = TAT I
+ = 1/1 RRM CONTROL

TIME [DAYS]

OPTICAL DENSITY [750 NM. / CM.]

Figure 2.
Figure 3.
DOLORES RIVER AT DOLORES
SEPTEMBER 8 1977

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

RELATIVE FLUORESCENCE X 30

TIME [DAYS]
Figure 5.

DOLORES RIVER AT DOLORES
NOVEMBER 29 1977

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

TIME [DAYS]
DOLORES RIVER AT DOLORES
NOVEMBER 29 1977.

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

OPTICAL DENSITY [750 NM. 1 CM.]

TIME [DAYS]

Figure 6.
Figure 7.

DOLORES RIVER AT DOLORES
NOVEMBER 29 1977

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

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

RELATIVE FLUORESCENCE X 30

TIME [DAYS]

Figure 8.
Figure 10.
Figure 11.

DOLORES RIVER AT DOLORES
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.

TIME [DAYS]

3.0 1.5 3.0 4.5 6.0 7.5 9.0 10.5 12.0 13.5
Figure 12.
Fig. 13. Dolores River at Dolores March 8, 1978. Table of optical density at 750 nm. 

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

Graph showing optical density changes over time in days.
DOLORES RIVER AT DOLORES
MARCH 8 1978

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

2.0  1.5  3.0  4.5  6.0  7.5  9.0  10.5 12.0 13.5
TIME [DAYS]

Figure 14.
DOLORES RIVER AT DOLORES.
MARCH 8 1978

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

Figure 15.
Dolores River at Dolores
March 8, 1978

H = TRT H
I = TRT I
+ = 1/2 ppm Control

Relative Fluorescence x 30

Time (Days)
Figure 17.

DOLORES RIVER AT DOLORES
MAY 10, 1978

A = TRTS. A, B, E, F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM CONTROL
DOLORES RIVER AT DOLORES
MAY 10, 1978

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

RELATIVE FLUORESCENCE X 30

Figure 18.
the system. Addition of phosphorus caused a slight growth increase averaging 16.7 mg/l VSS over an average of 5.9 mg/l VSS for the sample blank. Nitrogen and phosphorus addition increased the biomass to an average of 95.6 mg/l VSS substantiating the predicated nitrogen and phosphorus limitation.

The Dolores River sample at Dolores was found to be oligotrophic with respect to nutrients during the algal bioassay test period from September 1977 through May 1978. Both nitrogen and phosphorus must be increased by substantial amounts in order to change the degree of eutrophication.

Conclusions -

a. The sample was limited by both nitrogen and phosphorus.

b. There was a good correlation between chemical analysis and algal bioassay.

c. No metal toxicity was observed.

d. This sample represents an infertile body of water in terms of algae with oligotrophic to mesotrophic tendencies in the future.

2. Dolores River below Rico Tailings (Figures 19 - 22)

Dolores River above Rico Tailings (Figures 23 - 26)

Dolores River below West Dolores River (Figures 27 - 30)

These samples were subjected to algal bioassay only during September 1977. Consequently it was difficult to draw any conclusions beyond the limiting nutrient at that sampling date. Chemical analysis indicated a phosphorus limitation but as was true with the Dolores River at Dolores indigenous nutrient concentrations were extremely low. This fact made it appear likely that these sampling sites would be nitrogen limited as well.
Figure 19.

DOLORES RIVER BELOW RICO TAILINGS
SEPTEMBER 8 1977

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

OPTICAL DENSITY [750 NM, 1 CM]

TIME [DAYS]
DOLORES RIVER BELOW RICO TAILINGS
SEPTEMBER 8 1977

H = TRT H
I = TRT I
+ = 1/1 RAMP CONTROL

OPTICAL DENSITY [750 NM, 1 CM]

TIME [DAYS]

Figure 20.
DOLORES RIVER BELOW RFID TAILINGS
SEPTEMBER 8 1977

A = TRT B E F
C = TRT V D
D = TRT G + EDTA
G = I I ARM + EDTA
CONTROL

RELATIVE FLUORESCENCE X 30

Figure 21.

TIME (DAYS)

250
200
1750
1500
1250
1000
750
500
250
DOLORES RIVER BELOW RICO TAILINGS
SEPTEMBER 8 1977

H = TRT H
I = TRT I
+ = I/I AEM CONTROL

RELATIVE FLUORESCENCE X 30

TIME [DAYS]

Figure 22.
Figure 23.
Figure 24.
DOLORES RIVER ABOVE RICO TAILINGS
SEPTEMBER 8 1977

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
Figure 26.

DOLORES RIVER ABOVE RICO TAILINGS
SEPTEMBER 8 1977

H = TRT H
1 = TRT 1
+ = I/1 BAM CONTROL
DOLORES RIVER BELOW WEST DOLORES RIVER
SEPTEMBER 8 1977

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

OPTICAL DENSITY [750 NM. / CM.]
0.000
0.050
0.100
0.150
0.200
0.250
0.300
0.350
0.400
0.450

TIME [DAYS]
0.0
1.6
3.2
4.8
6.4
8.0
9.6
11.2
12.8
14.4

Figure 27.
DOLORES RIVER BELOW WEST DOLORES RIVER
SEPTEMBER 8 1977

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

RELATIVE FLUORESCENCE X 30

0 500. 1000. 1500. 2000. 2500.

0 1. 2. 3. 4. 5. 6. 7. 8. 9.

TIME [DAYS]

Figure 29.
DOLORES RIVER BELOW WEST DOLORES RIVER
SEPTEMBER 8 1977

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

Figure 30.
The results of the bioassay clearly indicated limitation by phosphorus and nitrogen with phosphorus being the most limiting. The maximum specific growth rates (Table 22) were much higher when phosphorus was added but due to the low nitrogen concentration growth was minimal when nitrogen also became limiting.

One interesting fact resulted from the bioassay. A metal toxicity problem was detected on the Dolores River above Rico Tailings. Growth did not occur when EDTA was removed from the system but biomass increased in the presence of EDTA.

Conclusions:

a. The samples were limited by both nitrogen and phosphorus.
b. There was a good correlation between chemical analysis and algal bioassay.
c. Metal toxicity was observed in the Dolores River above Rico Tailings but not at the other two sites.
d. During September 1977 these samples represented an infertile body of water.

B. Dominquez Project

Gunnison River near Grand Junction (Figures 31 - 44)

Chemical analysis was very accurate in predicating phosphorus limitation during November, 1977; January, 1978; and March, 1978. Bioassay verified that indigenous nitrogen was at a high enough concentration to support a large increase in biomass upon addition of phosphorus (Treatment C). Table 15 shows that the resulting biomass after addition of phosphorus alone nearly equaled the biomass increase when the sample was spiked with both nitrogen and phosphorus.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Limiting Nutrient(s)</th>
<th>Chemical Analysis</th>
<th>Bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>11/29/77</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gunnison River Near Grand Junction</td>
<td>Phosphorus</td>
<td>Phosphorus&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td><strong>1/9/78</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Gunnison River Near Grand Junction</td>
<td>Phosphorus</td>
<td>Phosphorus&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td><strong>3/8/78</strong></td>
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<td></td>
</tr>
<tr>
<td>Gunnison River Near Grand Junction</td>
<td>Phosphorus</td>
<td>Phosphorus</td>
<td></td>
</tr>
<tr>
<td><strong>5/10/78</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gunnison River Near Grand Junction</td>
<td>Phosphorus</td>
<td>Phosphorus&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Nitrogen also became limiting but only after significant growth occurs upon addition of phosphorus.

<sup>b</sup>No increase in growth occurred upon addition of both nitrogen and phosphorus over phosphorus addition alone. However, growth increased when total AAM was added indicating a possible trace metal limitation as well as phosphorus limitation.
GUNNISON RIVER AT GRAND JUNCTION
NOVEMBER 29 1977.

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

Figure 32.
Figure 33.
GUNNISON RIVER AT GRAND JUNCTION

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

RELATIVE FLUORESCENCE X 30

TIME [DAYS]

Figure 34.
GUNNISON RIVER AT GRAND JUNCTION
JANUARY 9, 1978

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

OPTICAL DENSITY [750 NM, 1 CM.]

A
C
D
G
+
CONTROL

TIME [DAYS]

Figure 35.
GUNNISON RIVER AT GRAND JUNCTION
JANUARY 9 1978

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

Figure 36.
GUNNISON RIVER AT GRAND JUNCTION
JANUARY 9 1978

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

RELATIVE FLUORESCENCE X 30

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

TIME (DAYS)

0.0 1.5 3.0 4.5 6.0 7.5 9.0 10.5 12.0 13.5

Figure 37.
GUNNISON RIVER AT GRAND JUNCTION
JANUARY 9 1978

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

Figure 38.
GUNNISON RIVER AT GRAND JUNCTION
MARCH 8 1978

A = TRT 5 A B C E
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 ARM + EDTA
CONTROL

OPTICAL DENSITY (750 NM, 1 CM.)

TIME (DAYS)
GUNNISON RIVER AT GRAND JUNCTION
MARCH 8 1978

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

OPTICAL DENSITY (750 NM. 1 CM.)

TIME (DAYS)

Figure 40.
GUNNISON RIVER AT GRAND JUNCTION.
MARCH 8 1978

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

RELATIVE FLUORESCENCE X 30
2250.
2000.
1750.
1500.
1250.
1000.
750.
500.
250.

TIME [DAYS]
0. 1. 2. 3. 4. 5. 6. 7. 8. 9.

Figure 41.
Figure 43.

GUNNISON RIVER AT GRAND JUNCTION
MAY 10, 1978

A = TRT S. A, B, E, F
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM CONTROL

OPTICAL DENSITY, [750nm, 1 cm]

TIME [DAYS]

0.000 0.050 0.100 0.150 0.200 0.250 0.300 0.350 0.400 0.450

0.0 1.5 3.0 4.5 6.0 7.5 9.0 10.5 12.0 13.5
GUNNISON RIVER AT GRAND JUNCTION

MAY 10 1976

A = TRT 5 A B E F
C = TRT <
D = TRT D
G = 1/2 RAM CONTROL

TIME [DAYS]

RELATIVE FLUORESCENCE X 30

Figure 44.
A slightly different situation arose in May, 1978. The sample was still phosphorus limited with treatments C and D nearly identical as far as biomass increase. However growth was retarded when compared to previous months and to treatment G (all AAM constituents added). This fact represents growth limitation by an element other than nitrogen or phosphorus. Further study of this water must be undertaken in order to ascertain the true cause of growth inhibition during May, 1978.

Conclusions:
1. Phosphorus was the limiting nutrient in all bioassays.
2. Chemical analysis and algal bioassay correlated well.
3. During the spring period another element in addition to phosphorus contributed to growth limitation.
4. This sample was classified as mesotrophic in its present state but a slight increase in the phosphorus concentration will result in a greater degree of eutrophication unless another element becomes limiting as was true in May, 1978.
5. No metal toxicity was observed.

C. San Miguel Project
1. San Miguel River near Placerville (Figures 45 - 62)

September, 1977 and November, 1977 chemical analysis indicated a tendency toward phosphorus limitation but due to the extremely low concentrations of phosphorus and nitrogen the bioassay verified, as expected, limitation by both elements. During this fall period the sample proved to be highly infertile but as the water level decreased by mid winter, the January, 1978 chemical analysis showed an increase in the nutrient concentration (Table 3). The bioassay reflected this higher nutrient
SAN MIGUEL RIVER AT PLACERVILLE
SEPTEMBER 8 1977

A = TRT A B C F
V = TRT C
D = TRT G
G = 1/1 AM + EDTA
CONTROL

OPTICAL DENSITY [750 NM, 1 CM]
SAN MIGUEL RIVER AT PLACERVILLE
SEPTEMBER 8 1977

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

0.0 1.6 3.2 4.8 6.4 8.0 9.6 11.2 12.8 14.4

TIME [DAYS]

Figure 46.
SAN MIGUEL RIVER AT PLACERVILLE
SEPTEMBER, 8 1977

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

Figure 47.
SAN MIGUEL AT PLACERVILLE
SEPTEMBER 8 1977

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)

Figure 48.
SAN MIGUEL RIVER AT PLACERVILLE
NOVEMBER 29 1977

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

0 2 4 6 8 10 12 14 16 18

TIME (DAYS)

Figure 49.
SAN MIGUEL AT PLACERVILLE
NOVEMBER 29 1977.

H = TRT H
I = TRT I
+ = 1/2 RAM 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 50.
Figure 51.

SAN MIGUEL RIVER AT PLACERVILLE
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

TIME (DAYS)
SAN MIGUEL RIVER AT PLACERVILLE

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
SAN MIGUEL RIVER AT PLACERVILLE
JANUARY 9 1978

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

OPTICAL DENSITY [750 NM, 1 CM]

0.000
0.050
0.100
0.150
0.200
0.250
0.300
0.350
0.400
0.450

TIME [DAYS]

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

Figure 53.
SAN MIGUEL AT PLACERVILLE

JANUARY 9 1978

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

OPTICAL DENSITY [750 NM. I CM.]

TIME [DAYS]
SAN MIGUEL RIVER AT PLACERVILLE
JANUARY 9 1978

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
SAN MIGUEL RIVER AT PLACERVILLE
MARCH 8 1978.

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

OPTICAL DENSITY [750 NM, I CM.]

0.450

0.400

0.350

0.300

0.250

0.200

0.150

0.100

0.050

0.000

0.1 1.5 3.0 4.5 6.0 7.5 9.0 10.5 12.0 13.5

TIME [DAYS]

Figure 57.
SAN MIGUEL RIVER AT PLACERVILLE
MARCH 8 1978

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

OPTICAL DENSITY (750 NM, 1 cm)

TIME (DAYS)
SAN MIGUEL RIVER AT PLACERVILLE
MARCH 8 1978

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

RELATIVE FLUORESCENCE X 30

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

TIME [DAYS]

Figure 59.
SAN MIGUEL RIVER AT PLACERVILLE
MARCH 8 1978

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

Figure 60.
SAN MIGUEL RIVER AT PLACERVILLE
MAY 10, 1978

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

Figure 61.
SAN MIGUEL RIVER AT PLACERVILLE
MAY 10 1978

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

Figure 62.
### Table 28
San Miguel Project
Limiting Nutrients

<table>
<thead>
<tr>
<th>Sample</th>
<th>Limiting Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chemical Analysis</td>
</tr>
<tr>
<td>9/8/77</td>
<td>San Miguel River near Placerville Phosphorus</td>
</tr>
<tr>
<td></td>
<td>San Miguel River near Sawpit Phosphorus</td>
</tr>
<tr>
<td></td>
<td>Leopard Creek Phosphorus</td>
</tr>
<tr>
<td>11/29/77</td>
<td>San Miguel River near Placerville Phosphorus</td>
</tr>
<tr>
<td></td>
<td>San Miguel River near Sawpit Phosphorus</td>
</tr>
<tr>
<td></td>
<td>Leopard Creek Phosphorus</td>
</tr>
<tr>
<td>1/9/78</td>
<td>San Miguel River near Placerville Phosphorus</td>
</tr>
<tr>
<td></td>
<td>San Miguel River near Sawpit Phosphorus</td>
</tr>
<tr>
<td></td>
<td>Leopard Creek Phosphorus</td>
</tr>
<tr>
<td>3/8/78</td>
<td>San Miguel River near Placerville Phosphorus &amp; Nitrogen</td>
</tr>
<tr>
<td></td>
<td>San Miguel River near Sawpit Phosphorus &amp; Nitrogen</td>
</tr>
<tr>
<td></td>
<td>Leopard Creek Phosphorus</td>
</tr>
<tr>
<td>5/10/78</td>
<td>San Miguel River near Placerville Phosphorus &amp; Nitrogen</td>
</tr>
<tr>
<td></td>
<td>San Miguel River near Sawpit Phosphorus</td>
</tr>
<tr>
<td></td>
<td>Leopard Creek Phosphorus</td>
</tr>
</tbody>
</table>

<sup>a</sup>Addition of phosphorus substantially increased the maximum specific growth rate, $\hat{\mu}_b$, indicating phosphorus limitation. However, due to the low level of both indigenous nitrogen and phosphorus growth was only minimal upon phosphorus addition as nitrogen became limiting as well.

<sup>b</sup>A trace element in addition to N and P was limiting.

<sup>c</sup>Trace metal toxicity was present at this sampling date.
concentration in a more fertile sample (VSS, Treatment A was 32.2 in January as compared to an average of 5.5 in September and November). However the nutrient increase was not proportional as the nitrogen concentration increased to a far greater extent than the phosphorus. The net result was a profound phosphorus limitation.

The March, 1978 bioassay showed a return to the fall conditions probably as a result of increasing water levels as the spring turnover began. Indigenous nutrients dropped again to very low concentrations and the sample again became nitrogen and phosphorus limited. As the spring turnover continued into May, 1978 chemical analysis showed the nutrient levels continuing to drop to extremely low concentrations. The May, 1978 bioassay reflected low nutrient concentrations with only minimal response even upon nitrogen and phosphorus addition (Treatment D). Other elements, as well as N and P, appeared to have become limiting on the San Miguel River at Placerville during May, 1978.

Conclusions:

a. The sample was limited by nitrogen and phosphorus during the fall and spring months but became phosphorus limited alone when the water flow and level were lower during winter.

b. Chemical analysis and bioassay correlated well.

c. During the spring period another element or elements in addition to N and P contributed to limitation.

d. No metal toxicity was observed.

e. This sample was classified as oligotrophic to mesotrophic.

The low nutrient concentrations indicate that both nitrogen
and phosphorus will be required before a shift in eutrophication occurs (graph, OD, 3/8/79 is not labeled).

2. San Miguel River near Sawpit (Figures 63 - 82)

As was true with most other samples analyzed during the bioassays, this sample appeared to be limited by phosphorus based solely on chemical analysis but in truth was limited by both N and P in all cases. Indigenous nutrient levels were low resulting in a low productivity sample which became productive only when nitrogen and phosphorus were introduced into the system.

The final bioassay in May, 1978 indicated a toxicity problem not before observed at this site. Growth upon addition of N and P (Treatment D) or total AAM (Treatment G) was not markedly greater than the sample blank. When this poor response was observed an EDTA spiked total AAM treatment (Treatment I) was added to the bioassay for this particular sample. The resulting growth with EDTA added was normal. Normally, as indicated by earlier bioassays, metal toxicity did not occur. It can only be assumed that spring turnover or runoff brought a toxic metal into the San Miguel River at the Sawpit site.

Conclusions:

a. The sample was limited by both nitrogen and phosphorus during each sampling period with phosphorus being the most limiting.

b. Algal bioassay and chemical analysis correlate well.
SAN MIGUEL RIVER AT SAWPIT
SEPTEMBER 8 1977

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

OPTICAL DENSITY [750 NM, 1 CM]

TIME [DAYS]
Figure 64
Optical Density (750 nm) vs. CM
SAN MIGUEL RIVER AT SAWPIT.
SEPTEMBER 8 1977

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

RELATIVE FLUORESCENCE X 30

TIME [DAYS]
SAN MIGUEL RIVER AT SAWPIT
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.)

TIME [DAYS]

Figure 67.
SAN MIGUEL RIVER AT SAWPIT
NOVEMBER 29 1977.

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

Figure 68.
SAN MIGUEL RIVER AT SAWPIT
NOVEMBER 29 1977

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

Figure 69.
SAN MIGUEL RIVER AT SAWPII

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

RELATIVE FLUORESCENCE x 30

TIME [DAYS]
SAN MIGUEL RIVER AT SAWPIT
JANUARY 9 1978

A = TRT A
B = TRT B
C = TRT C
D = TRT D
G = TRT G

OPTICAL DENSITY (750 NM. / CM.)

TIME (DAYS)

Figure 71.
SAN MIGUEL RIVER AT SAWPIT
JANUARY 9 1978

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

Figure 72.
SAN MIGUEL RIVER AT SAWPIT
JANUARY 9 1978

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

Figure 73.
SAN MIGUEL RIVER AT SAWPIT
JANUARY 9 1978

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)

Figure 74.
SAN MIGUEL RIVER AT SAWPIT
MARCH 8 1978

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

RELATIVE FLUORESCENCE x 30

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

TIME (DAYS)

Figure 77.
SAN MIGUEL RIVER AT SAWPIT
MARCH 8 1978.

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

Figure 78.
SAN MIGUEL RIVER AT SAWPIT
MAY 10, 1978

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

Figure 79.
SAN MIGUEL RIVER AT SAWPIT
MAY 10 1978

G = TRT. G
I = TRT. I [TRT. G + EDTA]
+ = 1/2 AAM CONTROL + EDTA
SAN MIGUEL RIVER AT SAWPIT
MAY 10, 1978

A = TRT 5 A, B, C, E, F
D = TRT D
G = TRT G
+ = 1/2 RAM CONTROL

RELATIVE FLUORESCENCE X 30

TIME [DAYS]

Figure 81.
SAN MIGUEL RIVER AT SAWPIT
MAY 10 1978

G = TRT. G
I = TRT. I [TRT. G + EDTA].
+ = 1/2 RAM CONTROL + EDTA

Figure 82.
c. Heavy metal toxicity was indicated during the spring possibly as a result of spring turnover.

d. An oligotrophic to mesotrophic condition can be expected at this site. Metal toxicity may play some role in this body of water remaining nonproductive.

3. Leopard Creek (Figures 83 - 100)

The conditions on Leopard Creek can be described in a very few sentences. Chemical analysis pointed in the direction of phosphorus limitation but upon closer examination it became obvious that this sample would be limited by both nitrogen and phosphorus simply because it contained such low concentrations of both. During the nine month period of the bioassays the highest of concentration of TSIN was 232 µg/l and of OP was 11. Both of these values occurred during the spring turnover, and even when the nutrient values were at their high point productivity was low.

Conclusions:

a. The sample was limited by both nitrogen and phosphorus during each sampling period with phosphorus being the most limiting.

b. Algal bioassay and chemical analyses correlated well.

c. No metal toxicity was observed.

d. This sample represents an oligotrophic body of water. Increased productivity is possible upon addition of nitrogen and phosphorus but substantial quantities of both would be necessary.
LEOPARD CREEK
SEPTEMBER 8 1977

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

OPTICAL DENSITY: [750 NM. 1 CM.]

0.000 0.050 0.100 0.150 0.200 0.250 0.300 0.350 0.400 0.450

TIME [DAYS]

0.0 1.6 3.2 4.8 6.4 8.0 9.6 11.2 12.8 14.4

Figure 84.
LEOPARD CREEK
SEPTEMBER 8 1977

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
LEOPARD CREEK
NOVEMBER 29 1977

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

Figure 87.
LEOPARD CREEK
NOVEMBER 29 1977.

H = TRT H
I = TRT I
+ = 1/2 RAM 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 88.
LEOPARD CREEK.
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

TIME [DAYS]
LEOPARD CREEK

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

Figure 90.
Figure 91.

LEOPARD CREEK
JANUARY 9 1978

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

CONTROL

OPTICAL DENSITY [750 NM, 1 CM]

TIME [DAYS]
LEOPARD CREEK
JANUARY 9 1978

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

OPTICAL DENSITY [750 NM] I <MJ>

0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000

TIME [DAYS]

Figure 92.
Figure 93.

LEOPARD CREEK
JANUARY 9 1978

A = TRTS A G
C = TRT C
D = TRT D
G = TRT G
+ = 1/2 AAM + EDTA
CONTROL
LEOPARD CREEK
JANUARY 9 1978

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

RELATIVE FLUORESCENCE X 30

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

0
3.0 1.5 3.0 4.5 6.0 7.5 9.0 10.5 12.0 13.5

TIME [DAYS]

Figure 94.
LEOPARD CREEK
MARCH 8 1978

H = TRT
L = 1/2 RM CONTROL

OPTICAL DENSITY 750 NM 1 CM
LEOPARD CREEK
MARCH 8 1978

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

Figure 97.
LEOPARD CREEK
MARCH 8 1978

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

Figure 98.
LEOPARD CREEK
MAY 10, 1978

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

OPTICAL DENSITY, [750 NM, 1 CM]

TIME [DAYS]
Figure 100.

LEOPARD CREEK
MAY 10, 1978

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

TIME [DAYS]

RELATIVE FLUORESCENCE X 30

2250.
2000.
1750.
1500.
1250.
1000.
750.
500.
250.
0.

1. 2. 3. 4. 5. 6. 7. 8. 9.
D. West Divide Project

Colorado River at Newcastle (upstream) (Figures 101 - 114)

Colorado River at Newcastle (downstream) (Figures 115 - 128)

The results of the bioassays on these two sites will be presented together because the two mimic each other so closely. The initial bioassay in November, 1977 indicated a tendency toward nitrogen limitation based on chemical analysis alone. However the bioassay procedure pointed in the opposite direction of an N and P limitation with phosphorus being the most limiting. Bioassays during the following months reflected a similar tendency of both nitrogen and phosphorus limitation and chemical analysis correlated a bit more closely than before.

Conclusions:

1. The samples were limited by both nitrogen and phosphorus during each sampling period with phosphorus being the most limiting.

2. Algal bioassays correlated well with chemical analysis except during November, 1977 when nutrient concentrations were so low an accurate assumption could not be made on chemical analysis alone.

3. No metal toxicity was observed.

4. At the present time this body of water would be classified as oligotrophic. The water does have a high growth potential as indicated by good response when nutrients are made available but addition of both nitrogen and phosphorus would have to occur in order for this to happen. In the short term future it appears this river will remain essentially the same.
### Table 29.
West Divide Project
Limiting Nutrients

<table>
<thead>
<tr>
<th>Sample</th>
<th>Limiting Nutrient(s)</th>
<th>Chemical Analysis</th>
<th>Bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/29/77</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Upstream)</td>
<td>Nitrogen</td>
<td></td>
<td>Nitrogen &amp; Phosphorus</td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Downstream)</td>
<td>Nitrogen</td>
<td></td>
<td>Nitrogen &amp; Phosphorus</td>
</tr>
<tr>
<td>1/9/78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Upstream)</td>
<td>Phosphorus</td>
<td></td>
<td>Phosphorus</td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Downstream)</td>
<td>Phosphorus</td>
<td></td>
<td>Phosphorus &amp; Nitrogen</td>
</tr>
<tr>
<td>3/8/78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Upstream)</td>
<td>Phosphorus &amp; Nitrogen</td>
<td></td>
<td>Phosphorus &amp; Nitrogen</td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Downstream)</td>
<td>Phosphorus &amp; Nitrogen</td>
<td></td>
<td>Phosphorus &amp; Nitrogen</td>
</tr>
<tr>
<td>5/10/78</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Upstream)</td>
<td>Phosphorus</td>
<td></td>
<td>Phosphorus</td>
</tr>
<tr>
<td>Colorado River @ Newcastle (Downstream)</td>
<td>Phosphorus</td>
<td></td>
<td>Phosphorus</td>
</tr>
</tbody>
</table>

*Addition of phosphorus substantially increased the maximum specific growth rate, \( \mu_b \), indicating phosphorus limitation. However, due to the low level of both indigenous phosphorus and nitrogen growth was only minimal upon phosphorus addition as nitrogen became limiting as well.*
COLORADO RIVER AT NEWCASTLE (UPSTREAM)
NOVEMBER 29 1977

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

RELATIVE FLUORESCENCE X 30

250.
2000.
1250.
1000.
750.
500.
250.

TIME (DAYS)

Figure 103.
COLORADO RIVER AT NEWCASTLE (UPSTREAM) NOVEMBER 29, 1977.

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
COLORADO RIVER AT NEWCASTLE, [UPSTREAM]
JANUARY 9, 1978

A = TRTS A B
B = TRT C
D = TRT D
G = TRT G
+
= 1/2 AAM + 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 105.
COLORADO RIVER AT NEWCASTLE [UPSTREAM]
JANUARY 9 1978

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

OPTICAL DENSITY [750 NM. I (CM.)

TIME [DAYS]

Figure 106.
COLORADO RIVER AT NEWCASTLE [UPSTREAM]

JANUARY 9 1978

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

Figure 107.
COLORADO RIVER AT NEWCASTLE [UPSTREAM]
JANUARY 9, 1978

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

RELATIVE FLUORESCENCE X 30

TIME [DAYS]

Figure 108.
Figure 109.

COLORADO RIVER AT NEWCASTLE [UPSTREAM]

MARCH 8 1978.

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

OPTICAL DENSITY [750 NM, 1 CM]

TIME [DAYS]
COLORADO RIVER AT NEWCASTLE (UPSTREAM)
MARCH 8 1978

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

Figure 111.
COLORADO RIVER AT NEWCASTLE [UPSTREAM]
MAY 10, 1978

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

Figure 113.
COLORADO RIVER AT NEWCASTLE [UPSTREAM].
MAY 10 1978

A = TRTSA B E F
C = TRTC
D = TRTD
G = TRTG
+ = 1/2 AAM CONTROL

Figure 114.

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

Figure 116.
COLORADO RIVER AT NEWCASTLE [DOWNSTREAM]

\[ H = TRT \ H \]
\[ I = TRT \ I \]
\[ + = 1/2 AAM CONTROL \]

**Figure 118.**
COLORADO RIVER AT NEWCASTLE, [DOWNSTREAM]
JANUARY 9, 1978

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

OPTICAL DENSITY (750 NM, I CM)

0.450
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000

TIME [DAYS]

Figure 119.
COLORADO RIVER AT NEWCASTLE [DOWNSTREAM]
JANUARY 9 1978

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

Figure 120.
COLORADO RIVER AT NEWCASTLE [DOWNSTREAM]
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]

Figure 121.
COLORADO RIVER AT NEWCASTLE (DOWNSTREAM)

JANUARY 9 1978

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

Figure 122.
COLORADO RIVER AT NEWCASTLE (DOWNSTREAM)
MARCH 8 1978.

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

Figure 123.
COLORADO RIVER AT NEWCASTLE [DOWNSTREAM]
MARCH 8 1978

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

OPTICAL DENSITY [750 NM, 1 CM]

TIME [DAYS]

Figure 124.
COLORADO RIVER AT NEWCASTLE (DOWNSTREAM)
MARCH 8 1978

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

Figure 125.
COLORADO RIVER AT NEWCASTLE (DOWNSTREAM)
MARCH 8 1978.

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

Figure 126.
COLORADO RIVER AT NEWCASTLE (DOWNSTREAM)
MAY 10, 1978

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

Figure 127.
COLORADO RIVER AT NEWCASTLE (DOWNSTREAM)
MAY 10 1978

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

RELATIVE FLUORESCENCE X 30

TIME (DAYS)
REFERENCES


