AN ALGAL AND CHEMICAL ANALYSIS OF EPH'S POND

by: matt murrell
E.S. 102
5/18/92
ART, DETHIER, EVANS
ALGAL AND CHEMICAL ANALYSIS OF EPH'S POND

I. INTRODUCTION: The goal of this experiment was to investigate the growth of algal populations in Eph's Pond in relation to the water chemistry over a period of several weeks. The hypothesis of the experiment was that, as time progressed, algal populations would flourish and correlations between algal growth and water chemistry could be drawn from experimental data obtained in the lab.

II. PROJECT DESCRIPTION and SAMPLING METHODS: This project was designed to analyze the algal growth and basic water chemistry of Eph's Pond over the period of April 28 to May 6. A set of six samples was collected on 4/28, 5/1 and 5/6 from representative sites throughout the pond. Along the south bank of the pond there were two, possibly three inlets noticed, and samples were collected at each of these. The fourth sample was collected approximately five feet from the concrete outflow pipe on the north bank of the pond which flows into the Iroosic. Samples five and six were collected at random sites along the north and northwestern banks of the pond. All samples, disregarding sample #4, were collected 10-12 feet from the bank at approximately four inches below the surface in open water. Sampling sites are elucidated in Figure #1.

III. LABORATORY TESTING PROCEDURES: Water samples collected at the pond were subjected to six procedures yielding twelve specific data groups:

1.) pH--measured with the pH meter
2.) ANC--measured using the pH meter according to guidelines specified in laboratory exercise #2.
3.) Ion Chromatograph: presence of the anions fluoride, chloride, sulfate and nitrate were tested using this apparatus.
4.) Atomic Absorption Spectrum: the presence of sodium and potassium were determined using this apparatus.
5.) Chlorophyll: total chlorophyll, as well as chlorophyll A and B were determined using the spectrophotometer on algal "mashes" in methanol and 80% acetone. Raw pond sample was filtered, and the filter was then ground in acetone and methanol. The filter was then centrifuged
out of the chlorophyll solution, and the absorbance of the solution was read at 645 nm and 663 nm. Equations, located in BIMO 322 Lab Experiment #5, were then used to calculate the amount of chlorophyll.

**CHLOROPHYLL A (ug/ml) = 12.7A_663 - 2.69A_645 **
**CHLOROPHYLL B (ug/ml) = 22.9A_645 - 4.68A_663 **
**TOTAL CHLOROPHYLL (ug/ml) = 20.2A_645 + 8.02A_663 **

Because only a portion of the raw sample was filtered the chlorophyll values within the entire sample volume were back-calculated using the volumes drawn through the filter (300/500 mL for samples #1-12, 200/500 mL for samples #13-18)

6.) Algal counts: an approximate number of algal cells within each of the collected samples was determined by preparing slides of filtered samples. Sample (5 mL) were drawn through grided filters which were allowed to dry. They were then mounted on slides with immersion oil and baked in the drying oven until the grids became translucent. Cover slips were then placed over the grids and a compound microscope was used to count the approximate number of cells per filter. Then the total number of cells was back-calculated, as in the chlorophyll counts, using the volumes filtered and the total raw sample volume.
IV. DATA/DISCUSSION: This section of the data discussion will be structured into two main parts: 1.) Ion concentrations: past vs. present 2.) Algal populations

1.) Ion Concentrations:

Testing was performed on Eph’s Pond during February ’89 in E.S. 102 as well as in the ’89 final project of Greg Balco. The values from both these investigations, located within Balco’s paper, are listed below.

*DATA COLLECTED 4/25/89*

<table>
<thead>
<tr>
<th>INLET #1</th>
<th>SAMPLE 1</th>
<th>Cl⁻</th>
<th>NO₃⁻</th>
<th>SO₄²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>105.6</td>
<td>16.0</td>
<td>31.6</td>
</tr>
<tr>
<td>SAMPLE 2</td>
<td>Off Scale</td>
<td>12.8</td>
<td></td>
<td>32.2</td>
</tr>
<tr>
<td>INLET #2</td>
<td></td>
<td>53.7</td>
<td>0</td>
<td>14.3</td>
</tr>
<tr>
<td>OUTLET</td>
<td>SAMPLE 1</td>
<td>60.8</td>
<td>0</td>
<td>20.6</td>
</tr>
<tr>
<td></td>
<td>SAMPLE 2</td>
<td>Off Scale</td>
<td>0</td>
<td>21.1</td>
</tr>
</tbody>
</table>

*DATA COLLECTED 2/89*

<table>
<thead>
<tr>
<th>INLET (average)</th>
<th>Cl⁻</th>
<th>NO₃⁻</th>
<th>SO₄²⁻</th>
<th>PO₄³⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>36.1</td>
<td>0</td>
<td>40.76</td>
<td>11.26</td>
</tr>
<tr>
<td>OUTLET (average)</td>
<td>105.3</td>
<td>0</td>
<td>14.00</td>
<td>0</td>
</tr>
</tbody>
</table>

The first point worthy of note is the presence of phosphate only at the inlets of the pond during the winter and the absence of phosphorus in April. This data suggests an important process occurring within the pond. This is the filtration capacity of the pond; 11.26 mg/L phosphorus came into the pond and, on average, 0 mg/L left the pond through the outlet. This filtering effect may have been due to the trapping of ions within sediment, for Eph’s Pond has as its floor a mud layer which reaches almost three feet below the bottom layer of water. Similarly, since phosphorus is valuable for living organisms, the decrease from the February inlet-outlet
as well as the decrease from February to April was most likely caused by 
organismic uptake. In April, phosphorous will probably already be 
absorbed by the time it reaches the pond inlet. During February, when 
there is little active vegetation around the pond, more phosphate would 
enter the pond and be used by whatever active life is present in the pond. 
Phosphorous may even tend to accumulate within the pond during the 
winter months and gradually disappear as pond life increases in the 
Spring.

The data collected during the present experiment indicates no 
phosphorous presence at inflows or outflows. Although Spring this year 
was approximately two weeks late enough life was present in the pond to 
expend whatever amount of phosphorous was present at one time.

Tables 1 and 2 indicate extremely high chloride, sulfate and nitrate 
values which were most likely due, according to Balco’s paper, to a 
structurally unsound sewage pipe leaking into the pond. Chloride, 
especially during February, was present in large amounts due to runoff 
from road salt around campus, for the drainage area for the pond 
encumbers nearby roads and parking lots from Cole Field to Route 2. 
Chloride values were less in the spring since the road salt input of chloride 
only exists as small amounts of remaining road salt running into the pond 
during rainfall. However, abnormally high values aside, the filtering 
capacity of the pond is once again indicated. Within both tables, the inflow 
of ion concentrations is much higher than the outflow concentration. 
Organistic and possibly sedimentary uptake of ions most likely produces 
this filtering effect.

Ion concentrations within this experiment were, in general, much 
lower than those recorded in ‘89. The primary cause for this was the 
repair of the sewer pipe. However, the data collected does exhibit the 
same filtering capacity of the pond. The data table is located on the 
following page.
DATA COLLECTED 4/28/92-5/6/92

<table>
<thead>
<tr>
<th></th>
<th>F-</th>
<th>Cl-</th>
<th>SO4</th>
<th>NO3</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>INLET*</td>
<td>#1</td>
<td>2.60</td>
<td>53.60</td>
<td>20.03</td>
<td>2.02</td>
<td>41.47</td>
</tr>
<tr>
<td>INLET</td>
<td>#2</td>
<td>2.80</td>
<td>45.60</td>
<td>18.68</td>
<td>1.21</td>
<td>32.93</td>
</tr>
<tr>
<td>OUTLET PIPE</td>
<td>2.00</td>
<td>36.60</td>
<td>17.37</td>
<td>1.16</td>
<td>22.33</td>
<td>3.73</td>
</tr>
</tbody>
</table>

(* indicates the major inlet of the pond; concentrations are higher here)

As is evident, Table 3 indicates the filtering effect of the pond on specific ion concentrations. Every ion, with the exception of fluoride (for which I had trouble acquiring credible values) undergoes a concentration decrease from the inlets to the concrete outlet pipe. This suggests that the pond still maintains a productive filtering mechanism, which could quite possibly be attributed in part to algal growth. As they increase in number, the algae remove ions from the water to be used for growth and various functional mechanisms. In this respect Eph's Pond may be considered a relatively healthy pond.

Tables 2 and 3 indicate that, passing from winter to spring, the concentrations of sulfate increase at the outlet and decrease at the inlets. During the winter when the ice shelters the pond from precipitation, acid rain being a contributor of sulfates, so the unfrozen inlets receive the precipitation and, hence, its contaminants; however, during the spring the pond receives direct precipitation so the inlets become less concentrated relative to the rest of the pond, which all flows through the outlet making it more highly concentrated. One reservation held about this hypothesis stems from the notion that if the inlets remain uncovered by ice during the winter the outlets will do the same, and will receive direct precipitation. This is incorrect. Since the outflow site is a pipe, the pond remains completely frozen over in that area, although the ice is somewhat thinner, therefore receiving no precipitation input. (I obtained empirical evidence of this fact during my Winter Study project in the form of wet feet and frozen jeans.) In Table 3 the highest filtering values occurred with
nitrate. Both sodium and chloride enter the pond almost exclusively through the inlets, so these ions are given more time to be used by organisms within the pond. Sulfate enters the pond mainly as precipitation in the form of acid rain; therefore, sulfate is not given as long within the pond to be used by organisms since it does not enter mainly through the inlet. I am unable to explain the low filtering capacity exhibited by nitrate.

The first correlation to be drawn from the Eph's Pond Data spreadsheet, located in the appendix, is between sodium and chloride. Although present naturally, these two ions often originate from the same man-made source, NaCl, which is present on Williamstown roads in large amounts during the winter months. Due to the fact that many of these roads and parking lots are within the drainage area for the pond, much of this salt makes passage through the stream. Figures #2, 3 and 4, especially #3 and #4, indicate the correlations between sodium and chloride on the three sampling dates. The overall sodium vs. chloride graph did not yield a high correlation value, yet the data within the specific sampling dates did. The values of .77709 and .99417 suggest that sodium and chloride did in fact originate from the same source at one time. The extremely heavy rainfall that occurred on 5/2, four days before the last set of samples were taken, probably washed any residual road salt remaining in the road or in the storm sewers into the pond. This explains the increase of average chloride concentrations from 33.70 mg/L and 40.33 mg/L on 4/28 and 5/1, respectively, to 62.66 on 5/6. However, the average sodium concentrations decreased, moving from 34.46 mg/L on 4/28 to 40.40 mg/L and 28.83 mg/L on 5/1 and 5/6. This decrease in concentration may be attributed to dilution, the addition of large amounts of water to the pond from excessive rainfall most likely diluted the sodium levels.

Nevertheless, the decrease does not disprove the hypothesis that both sodium and chloride in the pond originate from the same source, road salt. Had not sodium been input into the stream during the storm the concentrations would have fallen much more than they did. The sodium and chloride values from 5/6 very faintly conform to the "1.6 rule" which states that if sodium and chloride are originating from NaCl, then 1.6[Na]=2[Cl]. One of the better correlations is of data point #16: Cl=41.6 mg/L, Na=26.8 mg/L (26.8 x 1.6=42.88, close to 41.6). The variance from
this "1.6 rule" suggests most likely that chloride is entering the pond from an additional source other than road salt. The only possibility I can give would be runoff from the clay tennis courts; however, I do not know whether the courts contain any chloride or not.

The next several correlations are somewhat indirect in fashion. The first of these is chloride vs. sulfate, which should have no direct correlation. The correlations depicted in Figures #5, 6 and 7 indicate that chloride and sulfate either entered the pond from the same source or, more feasibly, entered by similar methods. Since acid rain is a major contributor to pond sulfate, and chloride enters the pond through ground flows washing road salts into the pond, the correlation (especially within the 5/6 data set) could be due to the rainfall. An analogous amount of sulfate may have entered the pond in the rain as chloride entered through ground flow. Figure #8 suggests similar reasoning. Sodium enters the pond just as chloride does, so the same explanation may apply to sodium vs. sulfate as chloride vs. sulfate. Another correlation, chloride vs. nitrate, indicates once again similar methods of entry into the stream. The main entry method for chloride is probably ground flow of road salt. Nitrate would enter the stream in much the same manner. It may be drawn from the soil during periods of precipitation or within basin areas in which water is flowing through the soil towards an input site (Eph's Pond). Therefore, the amount of ground flow due to precipitation will affect chloride and nitrate concentrations similarly. Figure #9 illustrates this correlation.
Figures:

5. CHLORIDE VS. SULFATE (4/29/92)

6. CHLORIDE VS. SULFATE (5/1/92)

7. CHLORIDE VS. SULFATE (5/6/92)
Figure #9

CHLORIDE VS. NITRATE

R = 0.87007
2.) Algal Populations: The abundance of algae within Eph's pond was investigated using two experimental laboratory procedures: 1.) the absorption of light to determine the amounts of chlorophylls within water samples and 2.) an approximation of the algal cell population size from slides prepared from grided filter paper. The hypothesis for this portion of the project was that the chlorophyll values (total, A and B) would correlate tightly with the algal cell approximations, for any increase in the cell population number, ideally, should translate into a higher chlorophyll content of the sample.

As indicated on the spreadsheet the algal population increased as expected from 4/28 to 5/1, but then plummeted unexpectedly on 5/6 to levels below those of 4/28. The hypothesis explaining the apparent decimation of the algal population involves no algal cell death but rather a massive dilution of the cells present in the pond. Although the pulse of water generated by the storm was most likely colder water containing high amounts of chloride, sodium, nitrate and whatever other substances it happened to acquire en route to the pond, this most likely did not kill the cells present. The sodium ion concentration decrease, discussed earlier, reinforces the dilution hypothesis. The concentration decreases of fluoride and sulfate also show indications of dilution.

<table>
<thead>
<tr>
<th>DATE</th>
<th>SO₄</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/28</td>
<td>19.05</td>
<td>2.66</td>
</tr>
<tr>
<td>5/1</td>
<td>19.91</td>
<td>2.70</td>
</tr>
<tr>
<td>5/6</td>
<td>15.55</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Although sulfate ions probably entered the pond by means of acid rain, the amount that entered in this manner was not proportional to the total amount of water that entered the pond during and after the storm; therefore, the ratio of sulfate ions to water fell, decreasing the concentration. A similar trend occurred within both the chlorophyll and algal cell population values. The actual amount of chlorophyll and algal
cells could have remained constant or even slightly increased during the period of apparent decrease, but the addition of vast amounts of water to the flow of the pond masked their true numbers. Values located table #5 indicate the dilution taking place within the chlorophyll and algal cell populations.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>ALGAL CELL #</th>
<th>CHLOR. A</th>
<th>CHLOR B</th>
<th>TOT. CHLOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/28:</td>
<td>2.4040 x 10^7</td>
<td>0.2935</td>
<td>0.4540</td>
<td>0.8071</td>
</tr>
<tr>
<td>5/1:</td>
<td>5.5617 x 10^7</td>
<td>1.0999</td>
<td>1.0460</td>
<td>1.7046</td>
</tr>
<tr>
<td>5/6:</td>
<td>2.3083 x 10^7</td>
<td>0.4700</td>
<td>0.5661</td>
<td>1.0359</td>
</tr>
</tbody>
</table>

All four data columns exhibit the same low-high-low value fluctuation produced by dilution. This suggests a strong correlation between the algal cell population and chlorophyll values. Figures #10, 11 and 12 show the correlations of chlorophyll A, B and total, respectively, with the algal cell number. Chlorophyll A shows the least correlation with an R value of .76295, followed by chlorophyll B with R=.81985. The total chlorophyll value, as expected, exhibits the strongest correlation to the algal cell number with R=.82617.

V. CONCLUSION: Eph’s Pond, overall, is a relatively “healthy” pond possessing a strong filtering capacity which allows it to maintain rather stable pH and ANC values throughout radical ion and biological variations. The large amount of limestone input into the pond gives the enormously large acid neutralizing capacity, which in turn maintains a somewhat basic pH regardless of other alterations of pond chemistry. The efficient flow through the pond also increases the “health” of the pond and its inhabitants. However, due to the nature of the pond’s drainage basin, possibility exists for major harm to come to the pond by way of road runoff, fertilizer contamination from lawns and playing fields, and any
sewage or similar pipe leaks like the one noted in 1989. The extremely shallow layer of water contained in the pond may be prone to damage, for it does not possess the potential for dilution a larger, deeper pond does. At present Eph’s Pond is a flourishing biological entity, but its location and physical characteristics may create problems in the future. Its filtering and buffering capacities are not limitless. In recognizing the “healthy” aspects of the pond and its dynamics, its vulnerabilities should be kept in mind and work should be done to maintain that “health.”
Figures:

*10 CHLOROPHYLL A VS. ALGAL CELL NUMBER

*11 CHLOROPHYLL B VS. ALGAL CELL NUMBER

*12 TOTAL CHLOROPHYLL VS. ALGAL CELL NUMBER
<table>
<thead>
<tr>
<th>Sample</th>
<th>Area (ft²)</th>
<th>Volume (gal)</th>
<th>pH</th>
<th>Hardness (ppm)</th>
<th>Alkalinity (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>1200</td>
<td>9600</td>
<td>7.5</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>Sample 2</td>
<td>1500</td>
<td>12000</td>
<td>7.2</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2000</td>
<td>16000</td>
<td>7.8</td>
<td>120</td>
<td>90</td>
</tr>
<tr>
<td>Sample 4</td>
<td>2500</td>
<td>20000</td>
<td>8.0</td>
<td>150</td>
<td>110</td>
</tr>
</tbody>
</table>

The pH levels range from 7.2 to 8.0, indicating a neutral to slightly alkaline environment.