RESEARCH PROJECT TECHNICAL COMPLETION REPORT

Institution  University of Connecticut  Date of Report  September 1978
OWRT Project No. A-075-CONN  OWRT Agreement No. 14-34-0001-8007
Project Title  A Bioassay Technique to Evaluate the Effect of Secondary Effluent on Algal Interactions in the Connecticut River
Principal Investigator(s)  F.R. Trainor, L.G. Harter
Project Began  October 1977  Project Ended  October 1978

PROJECT OBJECTIVES:

The main objectives of this research are:

1. Develop a bioassay technique which will yield qualitative and quantitative growth information about the effect of secondary effluent on algal interactions in a river system.

2. Compare laboratory and field methodology to show the influence of seasonal environmental factors on algal interactions in the Connecticut River.

ACHIEVEMENT OF OBJECTIVES:

Several goals have been accomplished during the study. First, a bialgal assay composed of two indigenous species representing two major algal groups in the Connecticut River has been developed. Second, the bialgal assay has been operated in the laboratory in dilute control medium, filtered river water and dilutions of secondary effluent. Simultaneously the assay has been incubated upstream and downstream from the Hartford sewage plant during a five day work week. Third, the choice of the green alga, Golenkinia sp., and the diatom, Cyclotella meneghiniana, enables in vivo and in vitro studies to be workable throughout the year. Fourth, the isolation of the blue-green alga, Oscillatoria sp., from the third major algal group has generated a trialgal assay.

Other endeavors concern the design of two computer programs to statistically evaluate the data. One program, a four-way ANOVA with interactions, analyzes the upstream and downstream river data from both the laboratory and field experiments. The second program, a simple ANOVA, includes the laboratory growth data obtained from effluent dilution experiments.
RESEARCH PROCEDURES USED:

The research operation has been divided into three phases. The first phase includes preliminary work to establish axenic unicellular cultures for assays. Phase II involves the implementation of algal assays in the field and in the laboratory. Phase III concerns the design of computer programs to evaluate the data. All phases have been accomplished.

1. Preparation of Pure Cultures

Ideally an algal assay should contain indigenous, axenic species. The green algae, *Golenkinia* sp., and the diatom, *Cyclotella meneghiniana*, have been selected as representatives of two major groups of algae (Chlorophyceae and Bacillariophyceae) present in the Connecticut River. Both species have been rendered axenic, i.e., free from bacteria, by plating and centrifugation techniques (23). Axenic cultures eliminate competition for nutrients by bacteria (7). The development of a control medium which duplicates nutrient levels found in the Connecticut River serves as a standard for comparing growth in river water and growth in effluent (Table I and References 2, 17, 18). Growth of the green alga and diatom together in the medium in the following cell number combinations: 1) the cell number of *Golenkinia* sp. greater than *C. meneghiniana* (*G> C-); 2) the cell number of *Golenkinia* sp. equals *C. meneghiniana* (*Go = Co); 3) the cell number of *Golenkinia* sp. less than *C. meneghiniana* (*G< C+) reveal compatible organisms (16). Establishment of a bialgal culture has enabled the introduction of a blue-green alga, *Oscillatoria* sp., representing the third major group (Cyanophyta) into the system. *Oscillatoria* sp. proves to be a compatible species, but viable only at certain temperatures.

2. Field and Laboratory Algal Assays

Field and laboratory studies have evaluated the effects of secondary effluent on bialgal interactions. Experiments conducted throughout the year have shown the impact of environmental factors on a bialgal assay. In addition a trialgal assay has been implemented over a winter - spring temperature regime.

a) Bialgal Assay

Laboratory and in situ studies have been conducted simultaneously over a five day work period. The week prior to the test period requires the transfer of cultures every two days to assure cells in an exponential growth phase (4). On the initial day of an experiment river water and secondary effluent are collected and sterilized by vacuum filtration through ultrafine (0.5um pore size) sintered glass filters. Preparation of the inoculum requires adjustment of cell numbers in the three combinations-- G+ > C-; Go = Co; G< C+. Laboratory studies necessitate simulation of river conditions through the use of rotated cultures, light cycles, controlled temperatures and daily nutrient replenishment (5). Field studies utilize dialysis sacs suspended in the river upstream (Site 1) and downstream (Site 3) from the effluent outfall (Figure 1 and Reference 19).
The cell number combination, Go = Co, is the best choice for an industrial or municipal laboratory application (Figures 2, 3 and Reference 22). The cell number combinations (G+> C- and G<- C+) exhibit diminished seasonal growth responses in the laboratory. In contrast laboratory algal interactions in the Go = Co combination closely mirror those found in the Connecticut River ecosystem. During algal bloom periods the in situ environment elicits more dramatic growth responses from the diatom in early spring and the green alga in summer (6). The diatom shows increased growth downstream (Site 3) throughout the year but most markedly at bloom periods, early spring and early fall (8,13). Laboratory growth experiments in 10% to 100% effluent dilutions in Site 1 filtrate show a more positive response of the green alga at 10% effluent concentration and the diatom at 40% effluent concentration (Figure 4 and Reference 20). Chemical analyses for phosphate and nitrate levels in the effluent have shown similar concentrations at different seasons (9,11, 12). Noticeable increased growth responses appear to result from seasonal fluctuations such as, ambient cell numbers in the water column, light and temperature (20). As more secondary effluent is added to the river a greater diversity of diatoms will prevail and is presently underway (communication with Hartford Water Pollution Control Plant).

b) Trialgal Assay

Compatibility studies in the control medium show Oscillatoria sp. can grow in mixed Golenkinia sp. and C. meneghiniana cultures (3). Upstream and downstream field and laboratory studies have been conducted in March and May. Overall better growth rates of the blue-green algae have been achieved in the May field experiments (1, 14). Oscillatoria sp. has exhibited little or no growth in the March field runs. Simultaneous laboratory experiments in March and May have yielded higher growth rates than the field runs. The substantially higher growth rates achieved at the controlled laboratory temperature, 21±1° C, suggest a limited temperature range over which Oscillatoria sp. is viable. Therefore, laboratory assays conducted at room temperature do not represent a true picture of algal interactions during cold in situ temperatures (10).

c) Design of Computer Programs

Two computer programs have been devised to analyze the data. One program, a four-way ANOVA with interactions, treats the upstream versus downstream data by means of an F-test (15). The F-test has emphasized the importance of initial cell numbers used in the inocula. The GoCo inocula cell densities have consistently yielded 95% confidence limits when the carrying capacity of the river is measured. The second program, a simple ANOVA, statistically evaluates the bialgal culture in effluent dilution experiments. The simple ANOVA affirms the importance of using the GoCo inoculum size as the practicable choice.
REFERENCES:


RESULTS OR CONCLUSIONS:

The most important accomplishments are:

1. The development of a bialgal assay which can measure the carrying capacity of a river ecosystem. This assay will show the effects of secondary effluent on two major algal groups (Chlorophyceae and Bacillariophyceae) in the Connecticut River.

2. The use of a trialgal or multiple algal assay which can illustrate qualitative and quantitative algal interactions during pollution problems.

3. The design of a computer program capable of evaluating the data.

LIST OF PUBLICATIONS:


ACKNOWLEDGMENT:

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Table 1. Composition of Harter Medium

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<th>Concentration (mg/l)</th>
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<td>NaNO₃</td>
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</tr>
<tr>
<td>K₂HPO₄</td>
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</tr>
<tr>
<td>MgSO₄·7H₂O</td>
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</tr>
<tr>
<td>Tris</td>
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<td>Na₂SiO₃·9H₂O</td>
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</tr>
<tr>
<td>B₁₂</td>
<td>3.0µg</td>
</tr>
<tr>
<td>Trace mix</td>
<td>10.0 ml</td>
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</tbody>
</table>

The trace mixture (21) contains:

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<td>Na₂MoO₄·2H₂O</td>
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Figure 1. Location of the Metropolitan District Sewage Treatment Plant and Sampling Sites (1-3)
Figure 2. Laboratory Growth of Bialgal Cultures in Site 1 and Site 3 Filtrates

KEY
- ● Golenkinia sp.
- □ Cyclatella meneghiniana
- Site 1
- Site 3
Figure 3. In situ Growth of Bialgal Cultures at Site 1 and Site 3

KEY
- Galenkina sp.
- Cyclotella meneghiniana
- Site 1
- Site 3

Cells/ml

Mar 1977 May Jun Jul Aug Sep May Feb Mar 1978
Figure 4. Growth of Bialgal Culture in Effluent/Site 1 Filtrates

KEY
- Galenkinia sp.
- Cyclotella meneghiniana
- 10% Effluent in Site 1
- 40% Effluent in Site 1