

Water Quality Assessment Experiment

Ecotoxicology is an extension of toxicology, the branch of biology that studies the ecological consequences of the effects of pollutants on organisms. With the right technology, the levels of different pollutants in soils or water can be measured quite precisely; however, determining the consequences of a particular or a combination of pollutants on organisms of the ecosystem is much more difficult. One widely used strategy to monitor the ‘health’ of an ecosystem is to monitor one or more organisms that are particularly sensitive to the presence of pollutants or other environmental disturbances. Organisms that are used in this way are called ‘**biological indicators.**’ An organism that is commonly used to monitor aquatic ecosystems is an algal species called *Pseudokirchneriella subcapitata*. (Notice that a scientific name is either italicized or underlined.) Recently the scientific name of this organism was changed, and it has previously been known as *Raphidocelis subcapitata* and *Selenastrum capricornutum*. These names should also be used when performing literature searches.

In this lab exercise you will be participating in a continuing Intro Biology investigation of the quality of water in local rivers. In general, rather sophisticated chemical analyses are necessary to determine which and if any chemical pollutants may be present in a body of water. By using *Pseudokirchneriella* as a bioindicator in a properly designed experiment, we can assess whether the properties of the water, in general, are deleterious to the growth of this and possibly other aquatic organisms. By determining the effects on the growth of *Pseudokirchneriella* in different water samples we can monitor changes in the water quality over time, and this is the objective of this exercise. Data that you collect this year will be combined with that collected in past and future years as an assessment of the quality of water. **Further important support information for this lab is available on the Water Quality Lab Web Resource Page, which can be found on the Biol 106 Home Page.**

Objectives of lab exercise

- 1) To learn about the use of bioindicator organisms as monitors of ecological health.
- 2) To improve your understanding of proper experimental design.
- 3) To learn effective strategies for searching the scientific literature.
- 4) To contribute to an ongoing study of the quality of water in local rivers.

What types of pollutants may affect water of local rivers?

There are many forms of pollution derived from human (and in some cases, natural) activities. Some types of pollutants that might affect water tested in this lab exercise are described below.

1. Acidic pH. There is great concern about the damaging effects of acid precipitation on aquatic and terrestrial ecosystems. Lakes, rivers and forests in the U.S., Canada, and Europe have suffered damage from rain, snow and fog. The acids arise from sulfur and nitrogen oxides in air emissions. Runoff from strip mines also can lead to acidification of waterways. Fish, invertebrates and microorganisms (such as *Pseudokirchneriella*) are all sensitive to the pH of the water in which they live.

2. Salinity. Elevated salt levels (NaCl and other inorganic substances) have a deleterious effect on many aquatic organisms. Salts enter aquatic ecosystems from numerous sources. Brine waste water is a byproduct of many oil wells. Salts leach into lakes and rivers from farmlands heavily laden with fertilizers and from roadways de-iced with salt.

3. Eutrophication. A eutrophier is a pollutant that provides excess nutrients to the aquatic ecosystem. The most common types of eutrophiers include untreated sewage and fertilizer run-off because both types contain a high nitrogen and phosphorous content. Normally, these chemicals act as limiting factors in an aquatic ecosystem. Present in excess, these nutrients cause an overgrowth of algae referred to as an “algal bloom.” When the algae die, their decomposition can lead to the death of aquatic animals.

4. Chemical pollutants. An environmental contaminant, or toxin, is a man-made chemical that has the ability to damage or injure organisms within an ecosystem. Environmental contaminants can come from a wide variety of sources (i.e. pesticides, sewage, herbicides, etc.) and can either directly kill an organism or move up the food chain through biomagnification.

Chemical pollutants typically can be placed into two categories: inorganic and organic pollutants. Inorganic pollutants include substances such as heavy metals (cadmium, lead, mercury, manganese) and salts (see above). Organic molecules are substances that contain carbon and hydrogen atoms, and there are many thousands of organic molecules produced through human industrial processes that can act as environmental toxins. Organic pollutants are the type of pollutant removed by the water filters containing “activated carbon” (aka “activated charcoal”) that people commonly install in their kitchen sinks.

The River Ecosystem Assessment Experimental Design

In this experiment you are going to examine a water sample from a local river for evidence of organic, inorganic or eutrophying pollutants. The basic experimental design will be as follows.

Pseudokirchneriella will be grown in the presence of:

- A. river water
- B. river water first treated with activated carbon
- C. spring water

The growth rate of *Pseudokirchneriella* (increase in number of cells over time) will be measured over a one week period, and the growth rates for the different water samples will be compared. Data for the entire class will be averaged, and differences between the growth rates for the different samples can be used to indicate the presence of the different types of pollutants. We will be applying the **Null Hypothesis** to this experiment; i.e., hypothesizing that there will be no detectable water pollution.

Three assignments are associated with this lab exercise – the Literature Research Assignment, Annotated Bibliography and the Lab Report – and are described below. Your group will also make an oral presentation to the class during the 3rd week of the lab exercise.

1. Literature Reference Assignment: Due Date _____

For this assignment, *each student* will prepare a bibliography of literature references. You should research this topic in the library and using on-line bibliographic databases.

Assignment Guidelines (see example reference list at end of this exercise):

A. Your references should include: (Sources on reserve are not acceptable. Do not use sources published before 1950.)

- ✓ 8 references from primary and secondary scientific literature (not books).
- ✓ 4 book references
- ✓ 3 references from tertiary scientific literature (not books)

B. References must be formatted as described in the document entitled *Guidelines for Writing Lab Reports*.

C. The references might pertain to the following topics:

- ✓ Biology and ecology of algae and *Pseudokirchneriella* (*Selenastrum*, *Raphidocellis*).
- ✓ Prior research using this algae as a bioindicator.
- ✓ Water quality studies of Ohio's rivers.
- ✓ Other bioindicators of aquatic ecosystems.
- ✓ Types and effects of chemical water pollutants.
- ✓ General characteristics of aquatic ecosystems.
- ✓ Caveats of bioindicators in environmental assessment.

D. Below each reference, indicate:

- ✓ whether it is a 1^o, 2^o, or 3^o source.
- ✓ how it was located; e.g., the index and key words used.
- ✓ the topic(s) to which it pertains.

You will be expected to locate and use information from some of these sources in your lab report. Any sources that are not available in Dawes library will need to be obtained through interlibrary loan (ILL). Since this takes between 2 - 3 weeks, you should send away for relevant literature sources ASAP after identifying the source. **Do not sign out any books from the library for this assignment. This inconveniences other students, and may result in a deduction to your grade.**

2. Annotated Bibliography Assignment: Due Date _____

For this assignment each student will prepare annotated bibliographies for 4 papers held on reserve in the library. The objective of this assignment is to teach you how to summarize scientific literature in the form of a short annotation, and to familiarize you with some other research that pertains to this lab exercise. You can select to annotate any four papers from the selection of papers placed on reserve in the library for this exercise.

The purpose of the annotation is not to describe the procedures and results of the paper in great detail, but rather to give an overview of the purpose, experimental strategy, types of experiments performed and results obtained, and the conclusions drawn. You should also state why the paper is relevant to this lab exercise. For this course we do not expect you to delve too deeply into the experimental procedures and specific results; however, some information about these should be presented. You are strongly encouraged to speak with your instructor should you have questions about the papers you choose to annotate. A reasonable length for an annotation is 300- 400 words.

Note: sample annotations are available on the Water Quality Lab Web Resource Page.

Procedure for River Ecosystem Bioindicator Experiment

This lab will take place over a 1 week period and will require you and your group to collect data each day for 8 days. This lab also requires that you keep a lab notebook in which you record all of your data, procedures and any other pertinent information.

Materials Needed:

(12) 50 ml flasks/ group (3 flasks per person)
Stock culture of *Pseudokirchneriella subcapitata*
Lab Notebook (discussed below)
water samples
filter apparatuses

Alga-Gro FW Medium
50 ml graduated cylinders
Pipets
Activated carbon

The Lab Notebook

A folder with notebook paper will be provided for you that will serve as your lab notebook. The lab notebook will be a valuable tool for you during this experiment and an important information source for the final lab report. It will be checked by your instructor. It will be your primary source of information when analyzing your results and writing your final lab report. Some general rules about keeping a lab notebook:

- ✓ Your notebook should begin with a **title** for the experiment and a **brief introduction** that includes the **purpose and hypothesis**. Also include **names** of all group members
- ✓ You should **record all procedures**. When procedures are repetitive, they can be recorded once and then referred to subsequently (e.g., “cell counts were performed as on 4/12”).
- ✓ **Do not rewrite** your lab notebook. A lab notebook must be the original record of what was done in the lab. It need not be immaculate in appearance, but it must be legible and coherent.
- ✓ Procedures should be clearly **dated**.
- ✓ Leave room to transcribe the data collected by your lab partners. There is no need to record all of the raw data from your partners, but space should be provided to include the average cell concentrations (cells/ml) for each flask on each counting day. You may wish to include a table (such as Table 1 below) for each counting day:

Table 1. Sample table for recording daily cell averages.

replicate #	Counts for each water sample (cells/ml)		
	untreated	treated	spring H ₂ O
1			
2			
3			
4			
Day Avg.			

Performing the Experiment

A. Obtaining and filtering the water samples.

Three water samples are present on the front bench: water from a local river, the same water containing activated carbon, and spring water.

1. Transfer 50 ml of each water sample to a graduated cylinder.
2. Using the funnel and filter paper provided, filter all the samples into clean flasks.

B. Setting up the water test samples.

Each student in your group will analyze each of the water samples. Each test will be performed in a 50 ml flask. Thus, if there are four students in your group, there will be 4 replicates for each water sample, requiring 12 flasks (Figure 1).

1. Clearly label all flasks with your group name, water type, replicate number and lab
2. For each test sample, combine in a sterilized 50 ml flask, the Alga-Gro FW medium, *Pseudokirchneriella subcapitata* and water sample to be tested in the following quantities.

9 ml of Alga-Gro FW Medium

(provides nutrients)

2 ml of *Pseudokirchneriella subcapitata*

9 ml of water sample

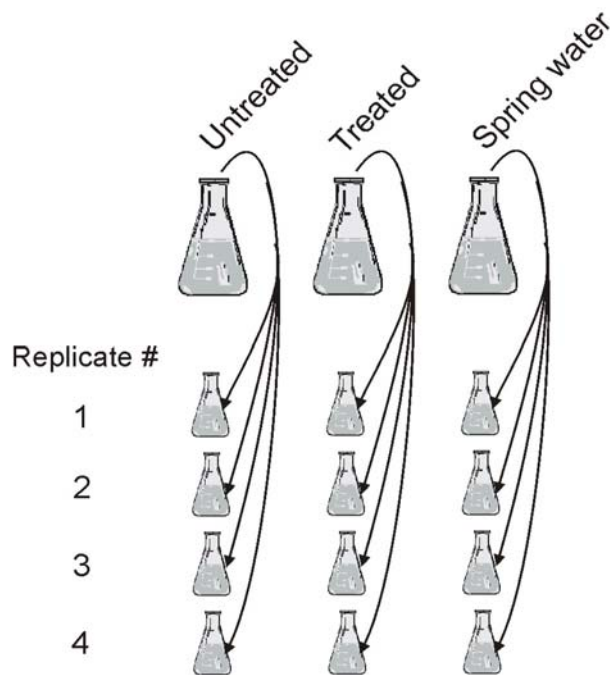
3. Gently swirl the samples to mix and cover the tops with foil.

C. Counting the Algae. Each group member will be responsible for counting cells for 3 flasks. The most reliable data would be obtained if students rotated the counting of different flasks. Why?

1. Draw a sample from flask #1 using a pipette and place on the hemacytometer plate.
(The lab instructor will demonstrate how to use the hemacytometer correctly.)
2. Adjust the microscope first using the 10X objective lens until you see the grid in the center, then switch to the 40X objective lens. *Pseudokirchneriella subcapitata* has a very distinctive crescent shape under a microscope which makes it easy to see. The appearance of the grid will be as shown in Figure 2.
3. A hemocytometer has two grids. You must count the cells in specific squares of both grids under the 40X objective lens, and then calculate the cell concentration. The drawing below shows the squares to be counted in one grid (the 4 corner squares and the center square). Thus, you will count cells in a total of 10 squares for each sample.
4. Use the following formula to calculate cell concentration:

$n/10 \times 250,000 = \text{cells} / \text{ml}$ where 'n' = the total number of cells in the 10 squares
The 250,000 factor represents the number of squares (which are actually cubes) in a ml.

Figure 1. Each water sample will be studied in replicate tests. Each test receives a sample of water to be tested, algae and Alga-Gro nutrients.



5. Average the data for all replicates that are receiving the same type of water.

For example: 1. $28/10 \times 250,000 = 700,000$

2. $18/10 \times 250,000 = 450,000$

3. $24/10 \times 250,000 = 600,000$

AVERAGE = 580,000 cells / ml

6. Repeat the above methods with the other flasks.

These same basic procedures should be followed on each counting day. On non-lab days, your counts will be made using microscopes and hemacytometers available in the Intro Biology lab.

Figure 2a. Appearance of the the hemacytometer.

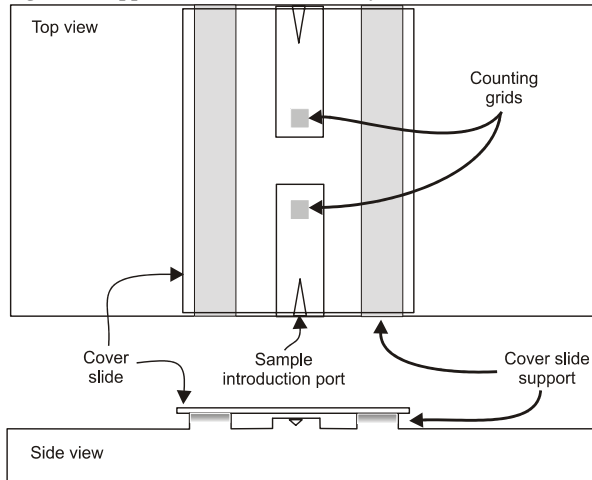
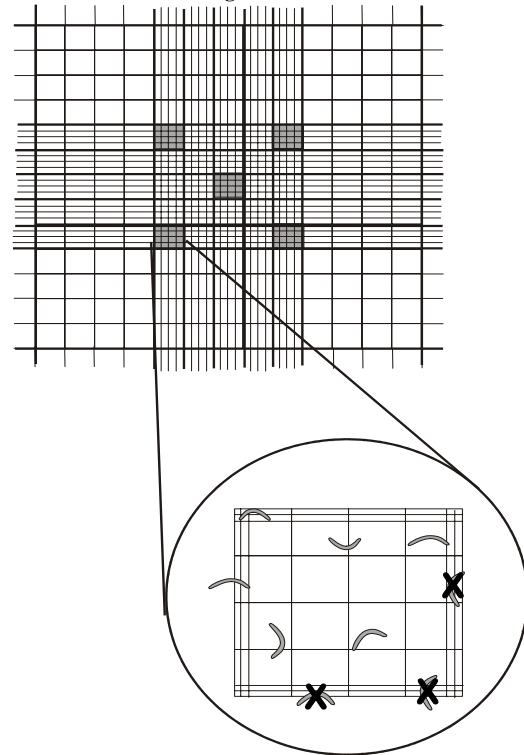


Figure 2b. Appearance of the counting grids of the hemacytometer. Count cells in the shaded squares located at center of the grid.



How to count cells that lie on the edges of squares:

Only count cells to the top and left touching the middle line. Do not count cells touching middle line at the bottom and right. Thus the correct cell count in the sample square is 6.

Data Analysis and Interpretation

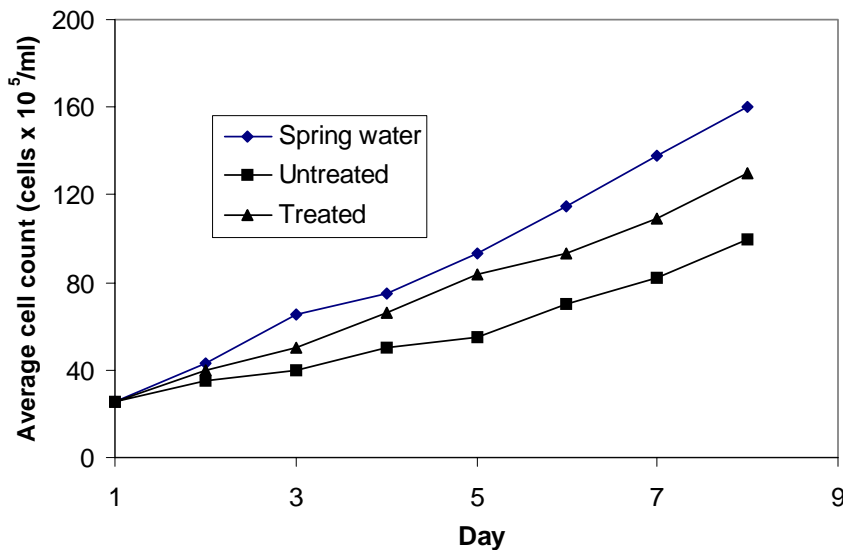
After counting the cells during the second week of this lab exercise, you will have 8 sets of data for each water sample. The data for each water sample should be placed into a summary table, as shown in Table 2.

Table 2. Sample table for recording cells counts.

Day	Avg. count for each water sample (cells/ml)		
	Untreated	Treated	Spring H ₂ O
1			
2			
3			
4			
5			
6			
7			
8			

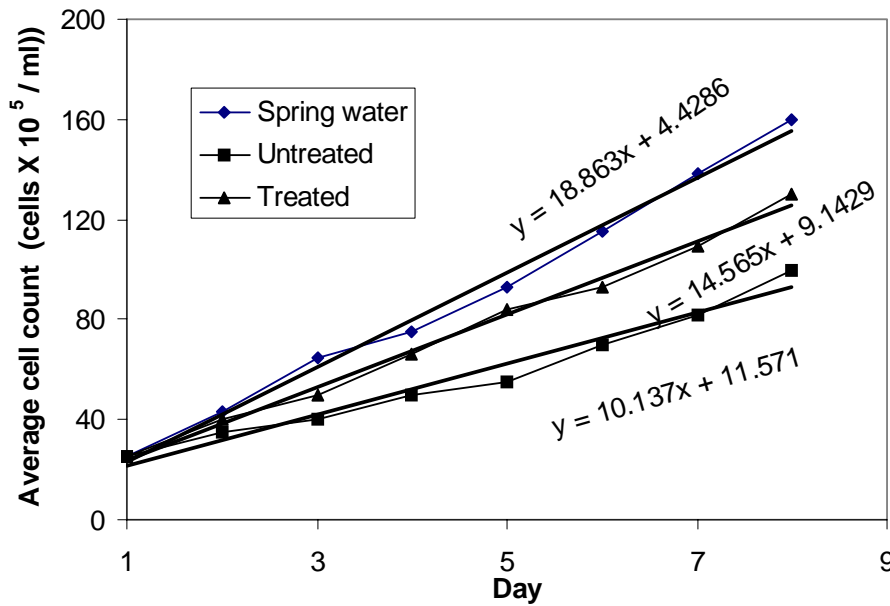
The average data should be graphed as shown for the hypothetical results shown below.

Figure 3 *Pseudokirchneriella* counts in 3 water samples.



From these data, trendlines can be drawn and slopes determined, as shown in Figure 4. If your data are not linear over the entire experiment, only draw the trendline through the linear segment.

Figure 4. *Pseudokirchneriella* counts in 3 water samples, with trendlines



From the slopes of the lines we determine the effect on the growth rate of *Pseudokirchneriella* of the treated and untreated water samples as compared to the control. The growth rates are determined from the slope of the lines. Remember that the equation of a line is ‘Y= mx + b’ and that ‘m’ is the slope. Thus, for the above data we obtain:

<u>Sample</u>	<u>Growth rate (10⁵ cells/ml/day)</u>
Spring water	18.9
Treated	14.6
Untreated	10.1

The effect of the water samples is calculated as “% inhibition” where:

$$\% \text{ inhibition} = 1 - \frac{\text{Rate}_{(\text{test sample})}}{\text{Rate}_{(\text{spring H2O})}} \times 100$$

Thus for the two water samples shown above:

$$\begin{aligned} \text{Treated \% inhibition} &= [1 - (14.6/18.9)] \times 100 = 23 \% \\ \text{Untreated \% inhibition} &= [1 - (10.1/18.9)] \times 100 = 47 \% \end{aligned}$$

The Lab Report: Due Date _____

The lab report for this exercise represents a significant percentage of your final grade – it should be given appropriate attention. Your lab report must be written by you alone– it is not part of the group project. It will be a full report, and you should review the *Guidelines for Writing Lab Reports* as you prepare each section. Some guidelines relevant to this particular assignment are presented below:

Introduction. The introduction should be well researched with information sources fully cited. Background Information of 3 - 4 pages is expected and should present information from the literature sources presented in your annotated bibliography and other sources.

Procedures. Fully describe the experimental procedure that your group used and how the results were calculated. The counting procedure need only be described once. Remember that the procedure should be adequate to allow someone else to repeat the experiment and get the same results.

Results. Tables and Figures. All must be fully and appropriately labeled.

- ✓ Include tables formatted as Tables 1 and 2 above presenting the cell concentrations (cells/ ml) calculated for each flask and the daily averages for all replicates. Do not include all of the counts made in the five hemocytometer squares (these should be in your lab manual.)
- ✓ Include a summary graph (similar to Figure 4 above) of the average cell concentration for each set of replicates plotted against day.
- ✓ Include a summary table of the class ‘% inhibition’ data similar to Table 3.

The data in the graphs should be fully and accurately described. Be sure to indicate any anomalies in the data. The ‘Description of Data’ section should also describe the ‘% inhibition’ values for your data and class averages.

Discussion.

Note: Examples illustrating potential results and their interpretations are presented on the Water Quality Lab Web Resource Page.

The results should be fully explained. The conclusions should identify which sample(s) studied by the class showed evidence of organic, inorganic or eutrophying pollutants. In the Explanation of Results section you should explain how you reached your conclusion. How do your data compare to the class averages, and why may there be differences? You should also discuss the results in context of literature sources, for example explaining why bioindicator results should be cautiously interpreted, and comparing class results to those of other published studies. The Explanation of Results section should be at least 2 pages in length.

Literature cited. Your report should include a minimum of 10 references, including the 4 papers that you annotated earlier this semester. This can include the lab manual, but only to reference the procedures, and the Web resource page. Other sources should be a selection of primary, secondary and tertiary sources. The references should be formatted as described in the general lab report guidelines.

Table 3. Sample table for recording group and class ‘% inhibition’ data

Replicate	Treated (% inhibition)	Untreated (% inhibition)
1		
2		
3		
4		
5		
6		
Average		

Here are two books on reserve that you can use as resources for your lab report (but not for the literature research assignment)

Cunningham WP, Saigo BW. 1997. Environmental science: A global concern. Dubuque, IA:
Wm. C. Brown Publishers,

Miller WE, Greene JC, Shiroyama T. 1978. The *Selenastrum capricornutum* Printz algal assay
bottle test: experimental design, application and data interpretation protocol. Corvallis,
OR: U.S. Environmental Protection Agency, 126pp.

Sample Reference List for Literature Reference Assignment

8 primary and secondary references: e.g.;

Fairchild JF, Ruessler DS, Haverland PS, Carlson AR. 1997. Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides. Archives of Environmental Contamination and Toxicology 32: 353 - 357.

Primary reference

Research Databases: Biological Abstracts and Biosis Previews

Key word: *Selenastrum*

Types and effects of herbicides on algae

4 book references: e.g.;

Shubert E. (ed) 1984. Algae as ecological indicators. London (GB): Academic Press, Inc.; 434pp.

MC Cat

Key word: algae

Information on using algae as bioindicators

3 tertiary references: e.g.;

Root M. 1990. Biological monitors of pollution. BioScience 40 (2): 83 - 86.

Research Databases: Biological Abstracts and Biosis Previews

Key word: biological monitors

Other bioindicators of aquatic ecosystems

ALL 15 OF THE REFERENCES YOU TURN IN MUST COME FROM SOURCES THAT ARE NOT ON RESERVE IN THE LIBRARY.

Articles on reserve for the annotated bibliography assignment (Biology 106)

*** 3 hour reserve, no overnight ***

- Baun A, Kloft L, Bjerg PJ, Nyholm N. 1999. Toxicity testing of organic chemicals in groundwater polluted with landfill leachate. *Environmental Toxicology and Chemistry* 18 (9): 2046 - 2053.
- Cheung KC, Chu LM, Wong MH. 1993. Toxic effect of landfill leachate on microalgae. *Water, Air and Soil Pollution* 69: 337 - 349.
- Fairchild JF, Ruessler DS, Carlson AR. 1998. Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor and metolachlor. *Environmental Toxicology and Chemistry* 17 (9): 1830 - 1834.
- Fairchild JF, Ruessler DS, Haverland PS, Carlson AR. 1997. Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides. *Archives of Environmental Contamination and Toxicology* 32: 353 - 357.
- Graffy EA. 1998. Low-level detection of pesticides ... so what? *Journal of Soil and Water Conservation* 53(1): 11 - 12.
- Lewis MA. 1990. Are laboratory-derived toxicology data for freshwater algae worth the effort? *Environmental Toxicology and Chemistry* 9: 1279 - 1284.
- Mayer P, Frickmann J, Christensen ER, Nyholm N. 1998. Influence of growth conditions on the results obtained in algal toxicity tests. *Environmental Toxicology and Chemistry* 17 (6): 1091 - 1098.
- Nyholm N, Källqvist T. 1989. Methods for growth inhibition toxicity tests with freshwater algae. *Environmental Toxicology and Chemistry* 8: 689 - 703.
- Pillard DA, DuFresne DL. 1999. Toxicity of formulated glycol deicers and ethylene and propylene glycol to *Lactuca sativa*, *Lolium perenne*, *Selenastrum capricornutum* and *Lemna minor*. *Archives of Environmental Contamination and Toxicology* 37: 29 - 35.
- Rojícková - Pádrtová R, Marsálek B. 1999. Selection and sensitivity comparisons of algal species for toxicity testing. *Chemosphere* 38 (14): 3329 - 3338.
- Root M. 1990. Biological monitors of pollution. *BioScience* 40 (2): 83 - 86.
- Turbak SC, Olson SB, McFeters GA. 1986. Comparison of algal assay systems for detecting waterborne herbicides and metals. *Water Research* 20 (1): 91 - 96.
- Walsh GE, Duke KM, Foster RB. 1982. Algae and crustaceans as indicators of bioactivity of industrial wastes. *Water Research* 16: 879 - 883.

YOU CANNOT USE ANY OF THESE REFERENCES FOR THE LITERATURE RESEARCH ASSIGNMENT.