Toxicity testing with green alga *Pseudokirchneriella subcapitata*

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Abstract

● Atrazine is herbicide that inhibits photosynthesis and interferes with other enzymatic processes. It is currently a pesticide of major concern due to its possible negative effects on health (nausea, dizziness, increased ovarian cancer risk) , aquatic organisms, levels in drinking water and the development of resistance in some weeds as they mutate

● The goal for this experiment was to test the growth inhibition of *Pseudokirchneriella (Selenastrum) subcapitata* algae exposed to atrazine using the Environmental Canada algal growth inhibition test via the 96-well microplate technique.

→ Eight concentrations of atrazine (ranging from 7.5-960 µg/L) were used with five replicates for each concentration with an initial cell density of 10,000 + 1000 cells/mL

● The endpoint for the reaction was absorbance of each well taken at 430 nm.

● The creation of a standard curve for cell concentration vs. absorbance was also a part of the experiment with five different cell densities ranging from 100 - $1x10^6$ cells/mL.

● The hypothesis was correct in ascertaining that cell density (as estimated by absorbance) decreased as a function of atrazine concentration.

Introduction

◙ Atrazine ($C_8H_{14}ClN_5$) is used extensively in Canada as a post-emergence weed control agent that targets the photosynthetic process. It has been banned in Europe.

◙ Atrazine degrades slowly in acidic waters and is considered to be a Priority A chemical for potential groundwater contamination → ranked as one of the 83 pesticides in the Agriculture Canada priority scheme for potential groundwater contaminants (Health Canada., 2011)

◙Chemical analysis is not enough to derive conclusions on the water quality of aquatic systems and thus bioassays can be a valuable complementary tool

◙ Objective: to determine the toxicity of atrazine on *Pseudokirchneriella subcapitata* growth by exposing the alga at the exponential growth phase to increasing concentrations of Atrazine

◙ Research Question: How does the concentration of atrazine affect the growth of *P. subcapitata*? Is absorbance a reliable estimate of cell density?

Hypothesis: As the concentration of atrazine is increased, we should see an increase in algal growth inhibition and hence lower cell densities.

Materials and methods

The Environment Canada 2007 protocol for toxicity testing using a green alga was applied to test the effects of a commercial formulation of atrazine.

Test organism: *Pseudokirchneriella subcapitata*
→ single culture purchased from Canadian Phycological Culture Centre and grown in Bold's modified basal medium (Sigma-Aldrich)
→ non-motile, unicellular, crescent-shaped green algae
→ model organism → readily available and easily cultured
→ moderately sensitive to toxic substances and no clumping thus ideal for enumeration

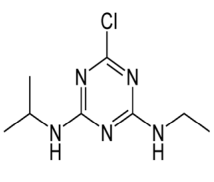

Figure 2: Atrazine structure

Figure 1: P. Subcapitata cells

Atrazine → Commercial formulation of atrazine used. Atrazine 480® containing 480 g/L atrazine + related triazines used.

Test solutions were prepared and dispensed in a predetermined pattern to in Wahtman 96-well polystyrene microplates.

◙Initial dose response experiments to determine range of toxicity for atrazine concentrations:

Number of concentrations: 8 → 7.5,15,30,60,120,240,480, & 960 µg/L

Number of replicates: 5 replicates/concentration
10 Blanks, 10 control

Each well received 200 µL of test solution, 10 uL of nutrient spike, and 10 uL of algal inoculum.

Incubation temperature: 24 ± 2◦C with continuous overhead "cool-white" fluorescent illumination with 56 ± 5.6 µmol m^{-2} s^{-1} for 72 hours

Endpoint: Cell yield (number of cells per mL) estimated by microscopy (Jena Zeiss at 100X magnification) using a hemocytometer, and absorbance at 430 nm

◙ Test methods were modified from protocol outlined here

Results

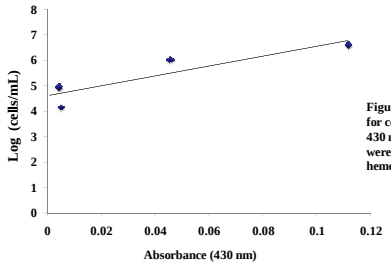

Figure 4: A first-order linear regression for cell concentration vs. absorbance at 430 nm for five serial dilutions. Cells were enumerated using a hemocytometer .

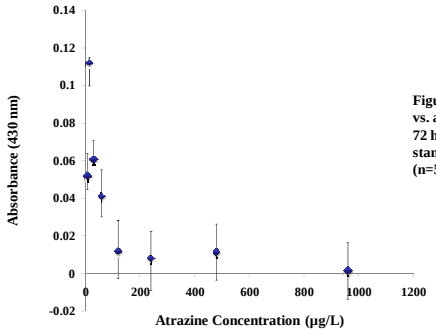

Figure 3: Absorbance (430 nm) vs. atrazine concentration after 72 hour exposure Error bars are standard errors of the means (n=5).

Discussion

● There was an obvious decline in absorbance as the atrazine concentration increased (Fig.2), and this decline seemed to be most significant up to 240 µg/L. Note: There were data points that did not follow the downward path such as the absorbance taken at 240 µg/L (Fig.2), where the absorption actually increased from the previous concentration.

● I did not find the standard curve for cell concentration vs. absorbance to be very successful. The variance in cells was greater than 10% for the initial solution of algae to be diluted and more cells needed to be counted to get a more accurate estimate of cell density.

● Overall, I think this protocol is fairly straightforward and efficient to use. For future work, one can continue to develop the standard curve, repeat experiment using florescence as an endpoint, assess toxicity of solvents such as methanol or acetonitrile to assess the maximum concentration of these solvents that can be used without affecting growth, so that the protocol may be repeated with an environmental sample.

References:

Biological test method. Growth inhibition test using a freshwater alga/ Method Development and Applications Section, Environmental Science and Technology Centre, Environment Canada. 2nd ed.c Her Majesty in Right of Canada(Environment Canada) 2006

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