Modeling the Impact of Dynamic Mixture Conditions: A Species Sensitivity Approach Using Freshwater Phytoplankton

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Introduction

- Pesticides from agricultural runoff often reach freshwater ecosystems, where they can impact critical primary producers such as algae
- Unlike single-pesticide studies, real-world exposure often involves mixtures
  - These mixtures can have synergistic, antagonistic, or additive effects
- Freshwater phytoplankton are highly diverse in their responses to pollutants, making them ideal indicators for understanding ecosystem-level impacts of chemical exposure
- Limited data exists on the sensitivity of different phytoplankton species to pesticide mixtures, hindering accurate ecological risk assessment
- Species sensitivity distributions (SSDs) aggregate chemical response endpoints across species, and are used as a tool for evaluating pesticide risks

Objectives

- Assess phytoplankton sensitivity to pesticide mixtures of copper (Cu), azoxystrobin (AZ), terbuthylazine (TER)
- Identify mixture interaction effects
- Determine the most sensitive freshwater microalgae

Methods

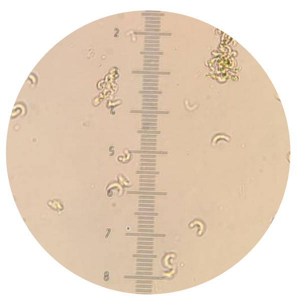
- Culture species in bioreactors until exponential growth phase
  - 15°C, 24 hour photoperiod
- Conduct 48 hr. growth inhibition test
  - OECD Guideline 201 – in 96 well microplates
  - Use pigment absorbance (ABS) as a proxy for cell abundance
  - Measure ABS with a spectrophotometer
- Calculate relative growth rate and model EC_{50}

Species

13 algae species cultured in bioreactors:

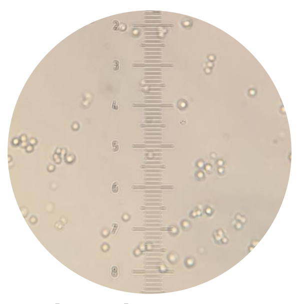
6 Green algae (40x)

*Raphidocelis subcapitata**

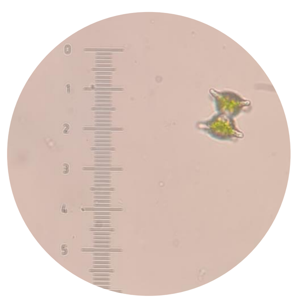


Actuodesmus obliquus

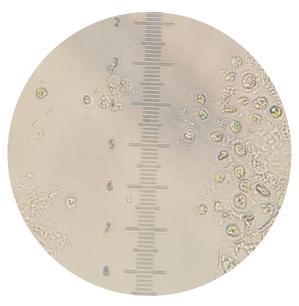
Chlorella vulgaris



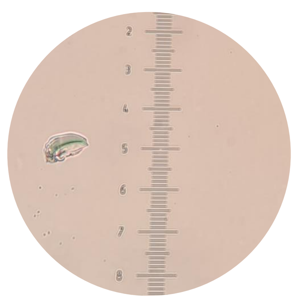
Staurastrum sp.



Chlamydomonas reinhardtii

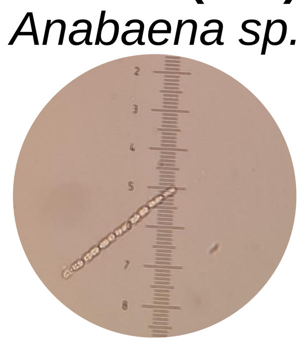


Rhodomonas lacustris

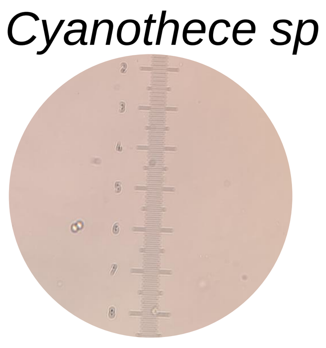


4 Cyanobacteria (40x)

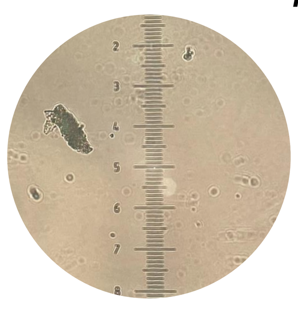
*Anabaena sp.**



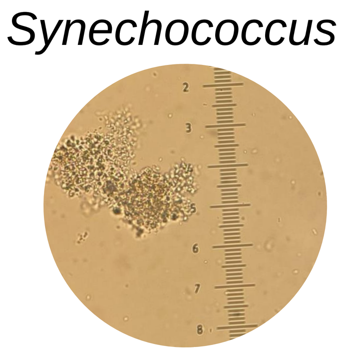
Cyanothece sp



Phormidium sp.

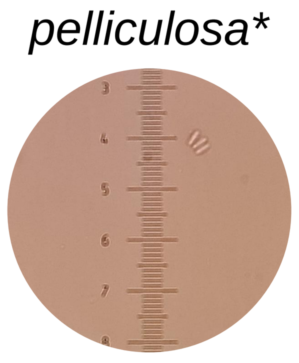


Synechococcus sp.

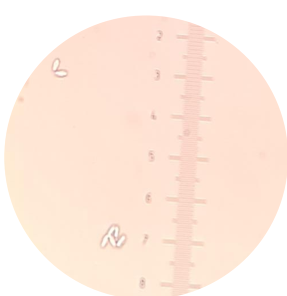


3 Diatoms (40x)

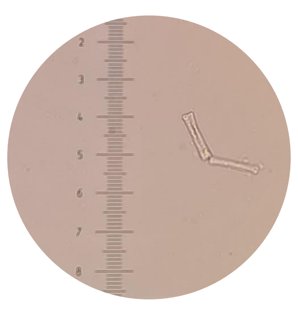
*Navicula pelliculosa**



Cyclotella sp.



Asterionella formosa



* Standard test species selected by OECD

Preliminary results: *Raphidocelis subcapitata*

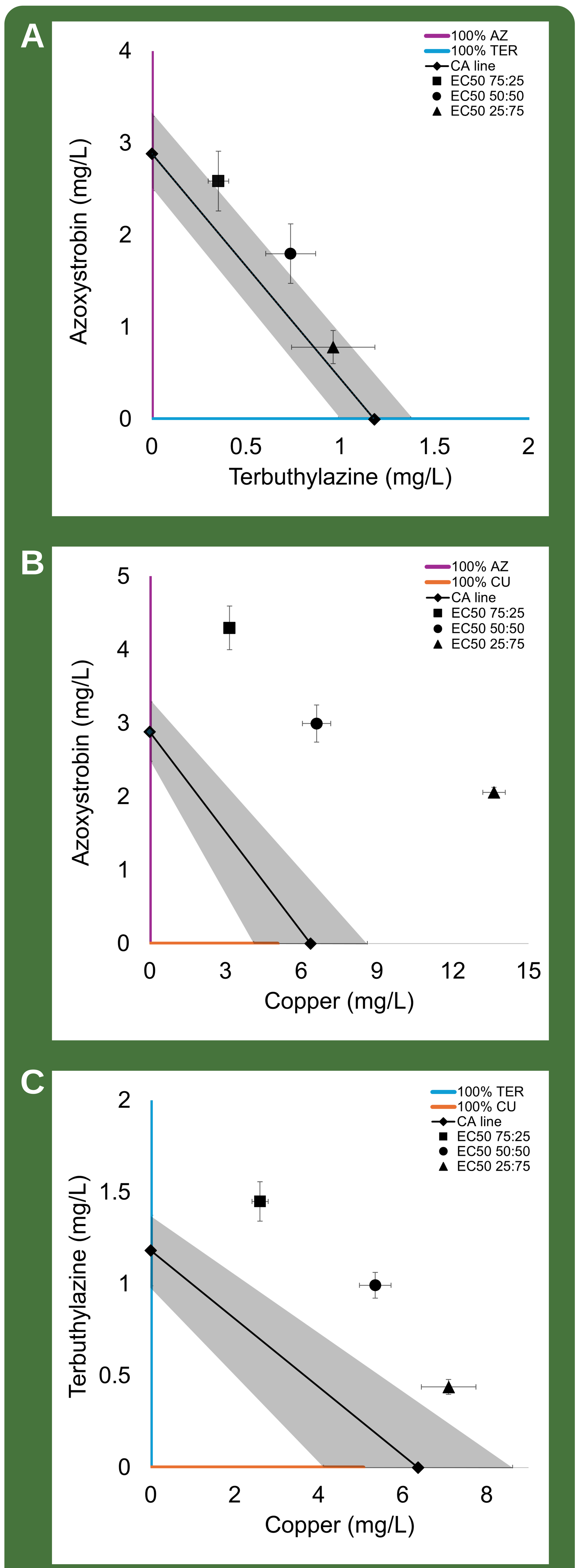


Figure 1. Isobolograms of binary chemical mixtures AZ:TER (A), AZ:CU (B), and TER:CU (C) for the species *Raphidocelis subcapitata*. The figures show the concentrations tested in the colored lines on both axes. The EC_{50} values of the relative growth rate of the ratios 25:75, 50:50, 75:25, and pure chemicals are shown in the points, with their standard error. The solid black line is the concentration addition (CA) line, with 95% confidence interval highlighted in grey. If the EC values are below the CA line, then there is a synergistic effect, and if they fall above the line there is antagonism.

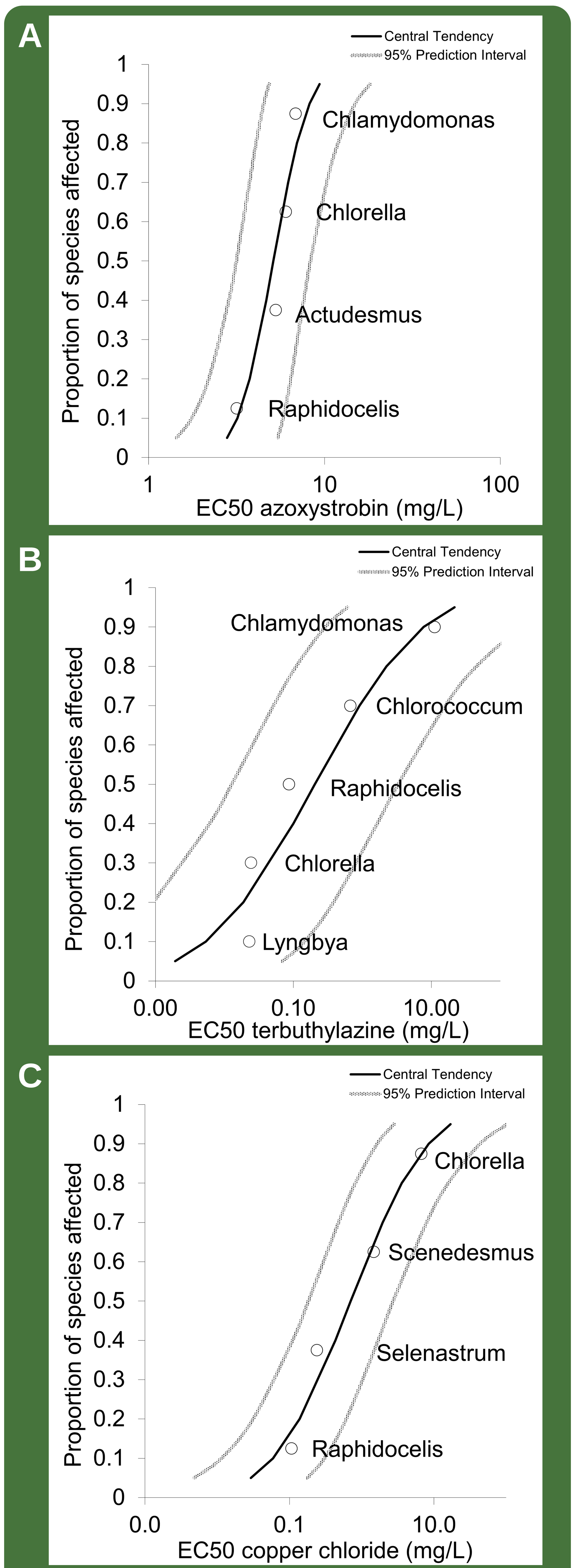


Figure 2. Species sensitivity distributions (SSDs) aggregate chemical response endpoints across species, and are used as a tool for evaluating pesticide risks. These supplemented SSDs are for azoxystrobin (A), terbuthylazine (B), and copper chloride (C). Data is supplemented with EC_{50} values from the US EPA ECOTOX Knowledgebase. Genus names are used but may have other species included.

Conclusion

- The two organic pesticides, azoxystrobin and terbuthylazine have an additive effect on the freshwater algae species, *Raphidocelis subcapitata*
- The organics mixed with the metal copper, have an antagonistic effect, but copper bioavailability must be measured to improve accuracy of test
- The development of freshwater algae SSDs need more data

Future plans

- Continue to test mixture experiments on other species
- Retest dose response assays for single compounds for more datapoints in SSD
- Measure the bioavailable fraction of copper in test systems
- Test chemicals in pulse exposures with and without time for recovery in between

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