



1ST INTERNATIONAL CONFERENCE ON RISK ASSESSMENT
OF PHARMACEUTICALS IN THE ENVIRONMENT
SEPTEMBER 8-9, 2016 | PARIS - FRANCE

Analysis of toxicological effects of atenolol, caffeine, lidocaine and oxytetracycline on *Raphidocelis subcapitata* and *Chlorella vulgaris* algae

**Authors: Zaniel Procopio, Dr. Colin Hunter, Professor Ole Pahl
Glasgow Caledonian University, Glasgow/UK**

Introduction

- There is an increasing concern about the constant emission of pharmaceutical residues (PR) in the hydric resources.
- These compounds are present in a range of anthropogenic products or natural substances.
- The aim of this research was to understand the biological effects caused by four drugs, individually, within controlled aquatic systems using algae cultures as bioindicators.
- Algae were selected as bioindicators in the toxicity tests, since they can generate an important characterization of acute and chronic effects of various toxic agents.



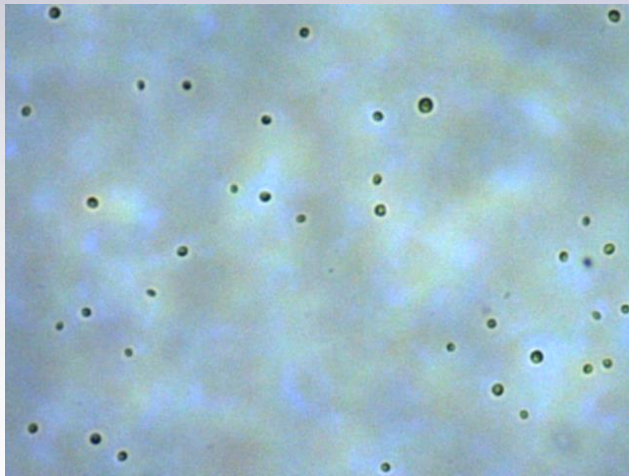
Introduction

- The following criteria were considered to select the algae used in this assay
 - Significant ecological representation within biocoenosis;
 - Knowledge of biology, physiology and eating habits;
 - Genetic stability and uniformity of the population;
 - Food chain importance;
- To address the potential toxicity of the drugs the Photosynthesis efficiency (PE) was evaluated; cells size and growth capacity were also quantified due to assess an overall effect based on the culture measurements of inhibition and/or stimulation.

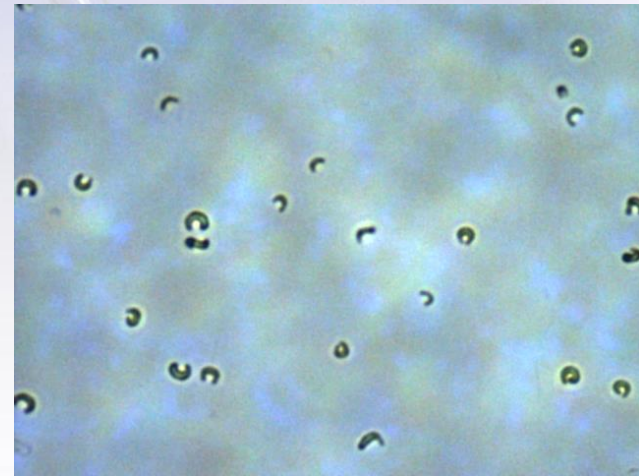


Methods

- Two algae were chosen to evaluate the possible effects of effluents containing different drugs concentrations;



Chlorella vulgaris



Raphidocelis subcapitata

- Algae were pre-cultured in Jaworski's Medium (JM) according the Culture Collection of Algae and Protozoa (CCAP) protocol.

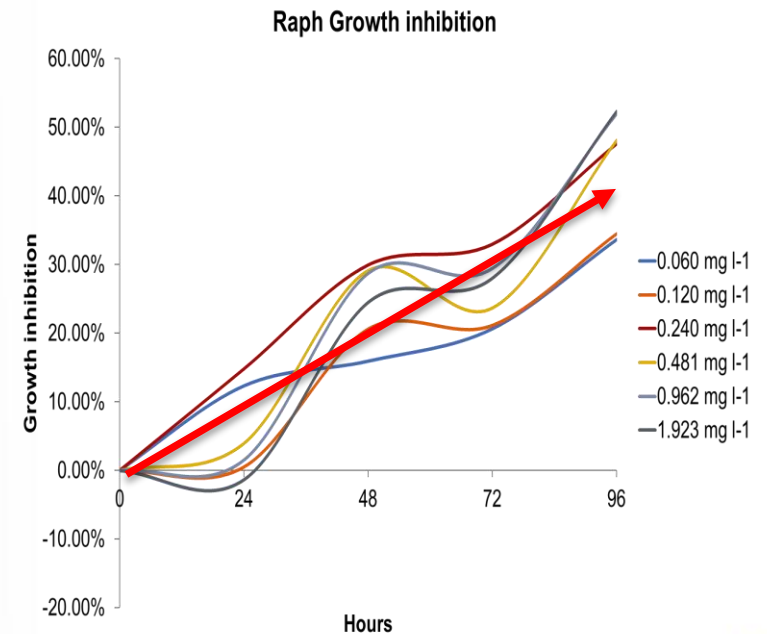
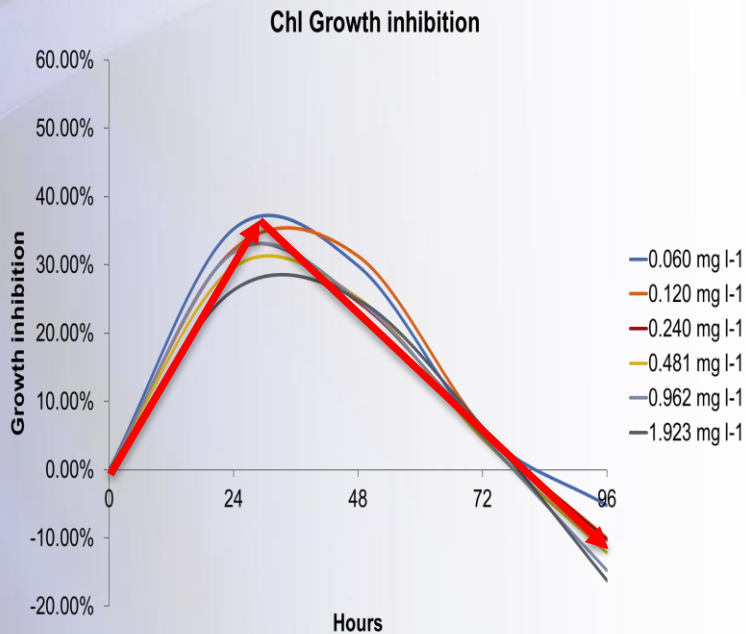
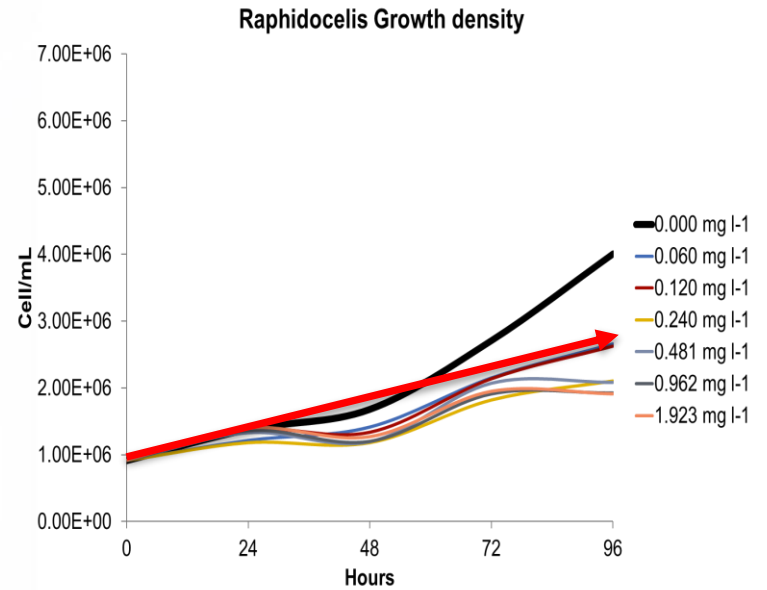
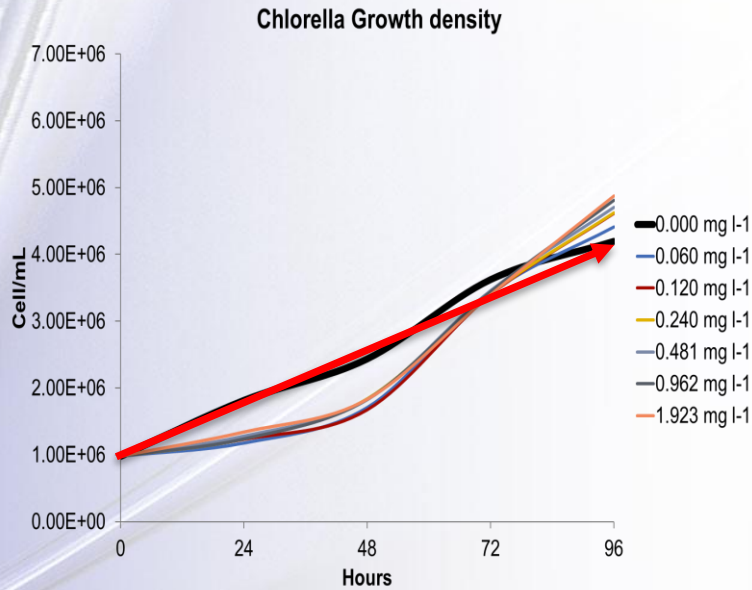


Methods

- Synthetic wastewater was prepared as described in the OECD guidelines. All experiments were performed in a test solution containing 90% JM and 10% of synthetic wastewater (v/v);
- PE was analysed after drug incubation, employing the ToxY-PAM Analyzer, cells sizes and concentrations were investigated with the Celeromics Micro Counter 1100.
- The election of pharmaceuticals is based on a prior study developed by Pills project (2012) adopting four key parameters: usage, excretion rate, removal in WWTP and Predicted No Effect Concentration (PNEC);
- Drug toxicity effects were tested in triplicate at six different concentrations between 0.003 & 2.000 mg l⁻¹; and all substances, individually over a period of 96 hours, in a controlled environment.

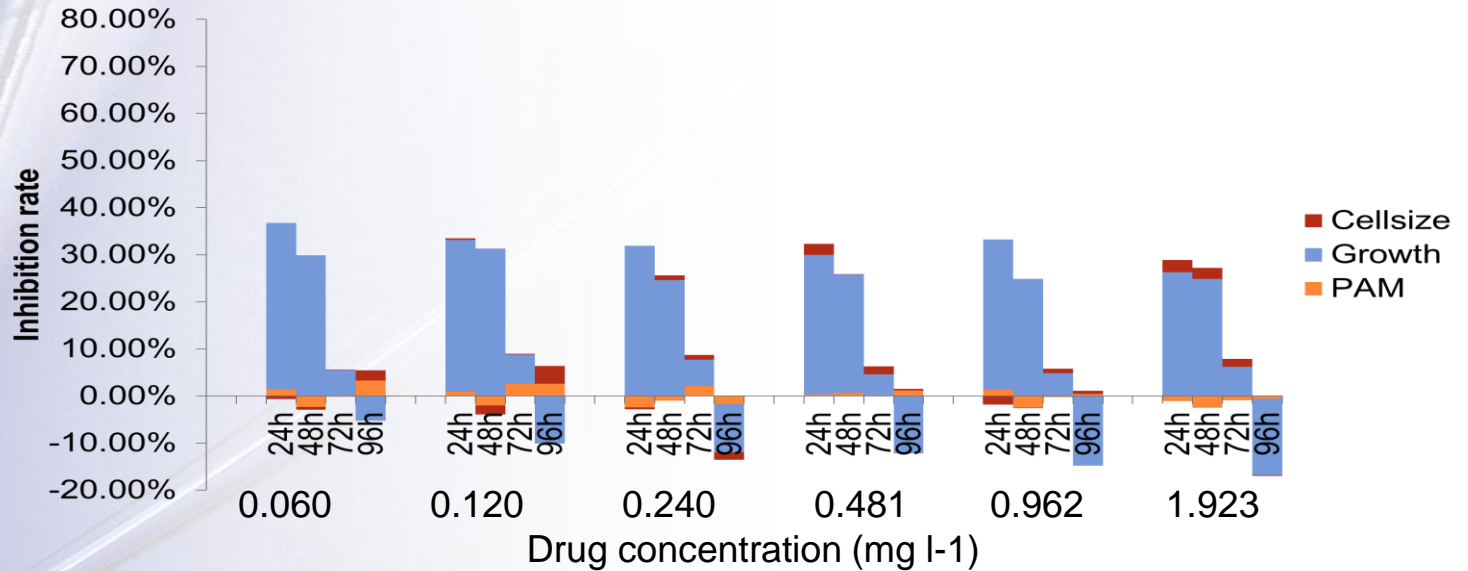


Results: Lidocaine effects on algae culture

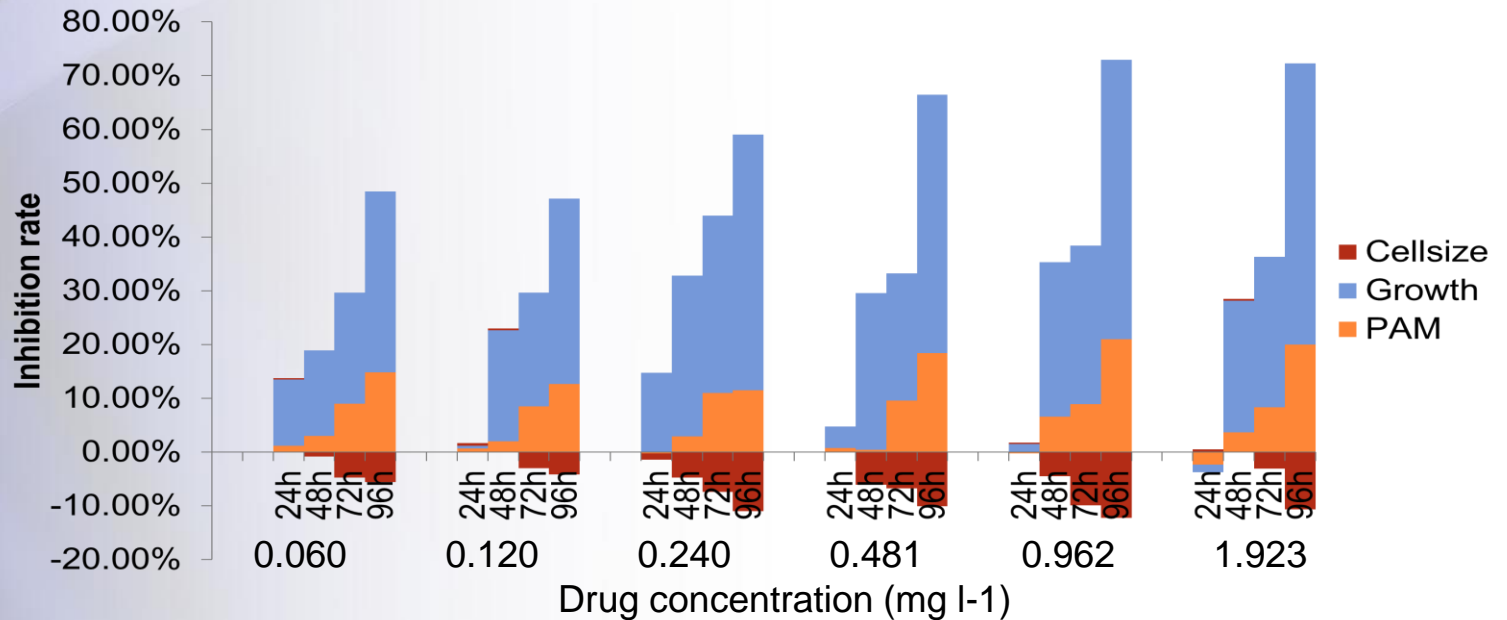


Results: Lidocaine effects on algae culture

Chlorella PAM, Growth and Cell size analyze

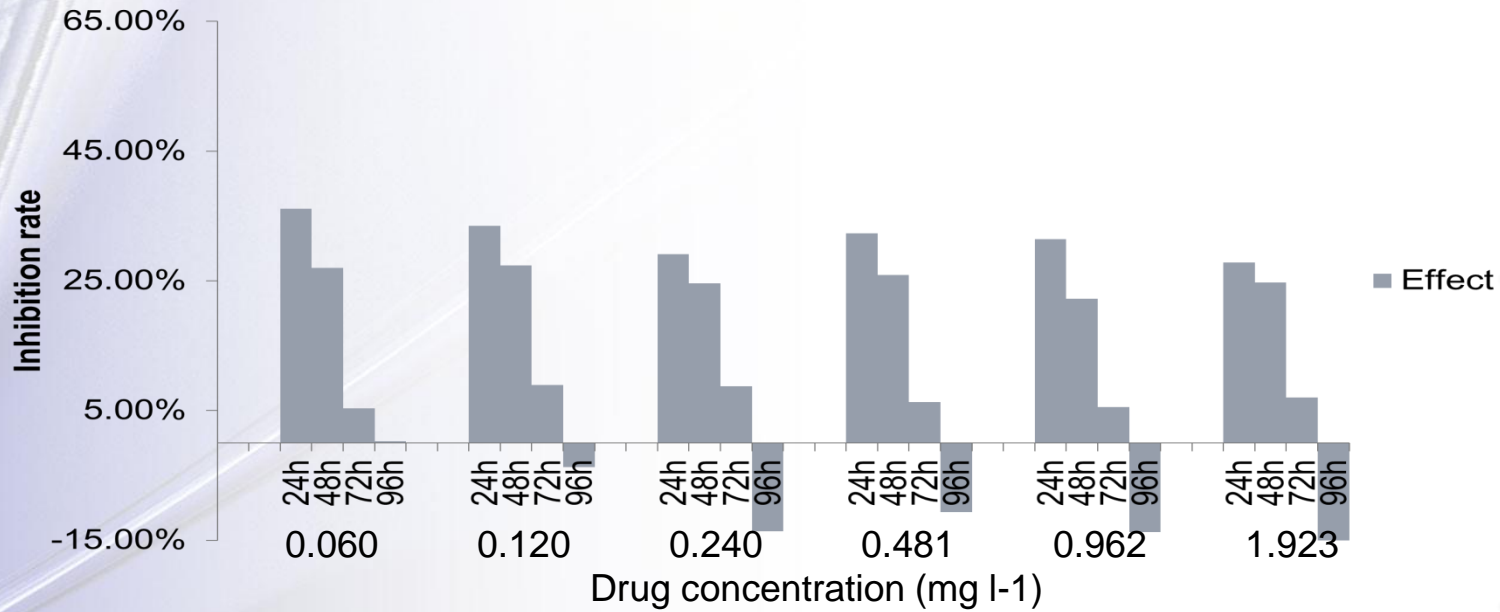


Raphidocelis PAM, Growth and Cell size analyze

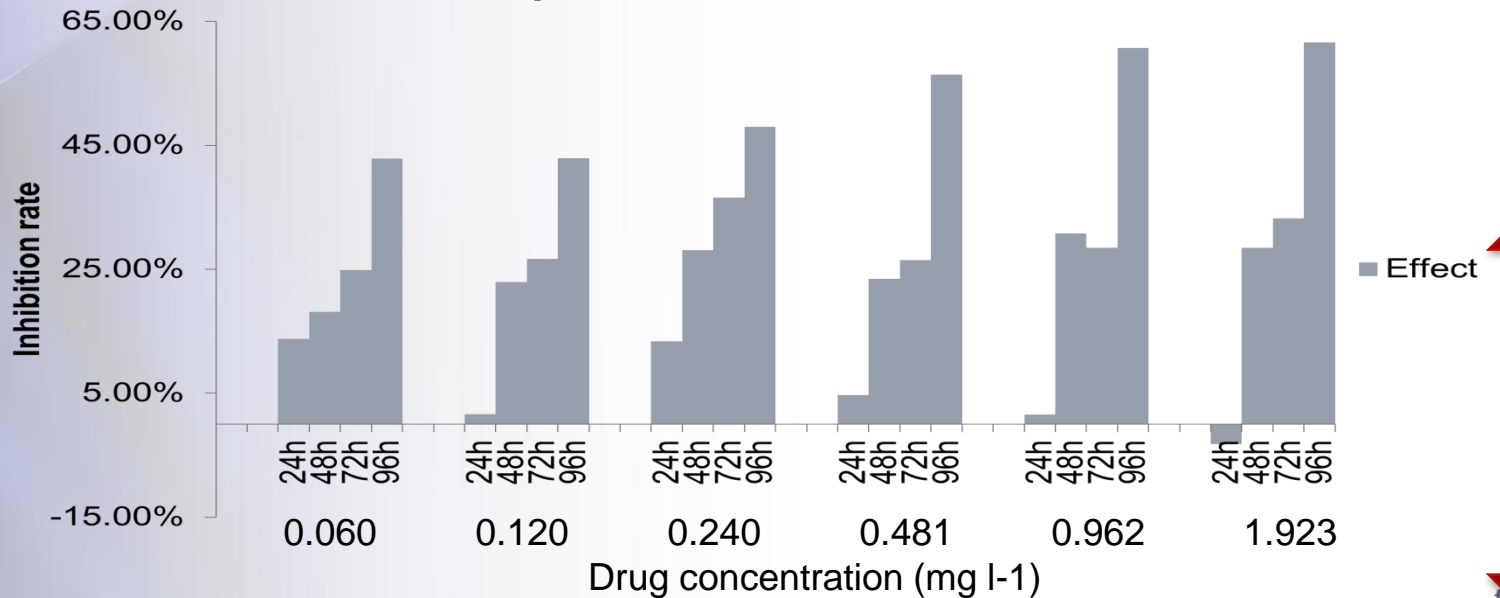


Results: Lidocaine effects on algae culture

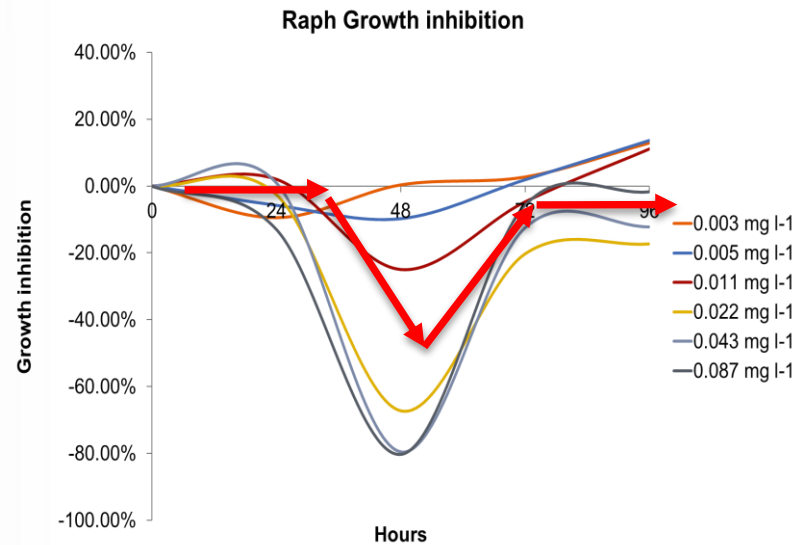
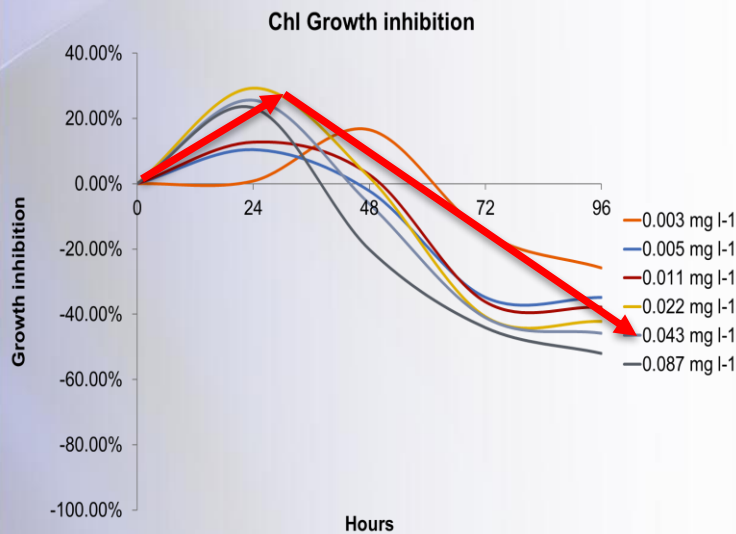
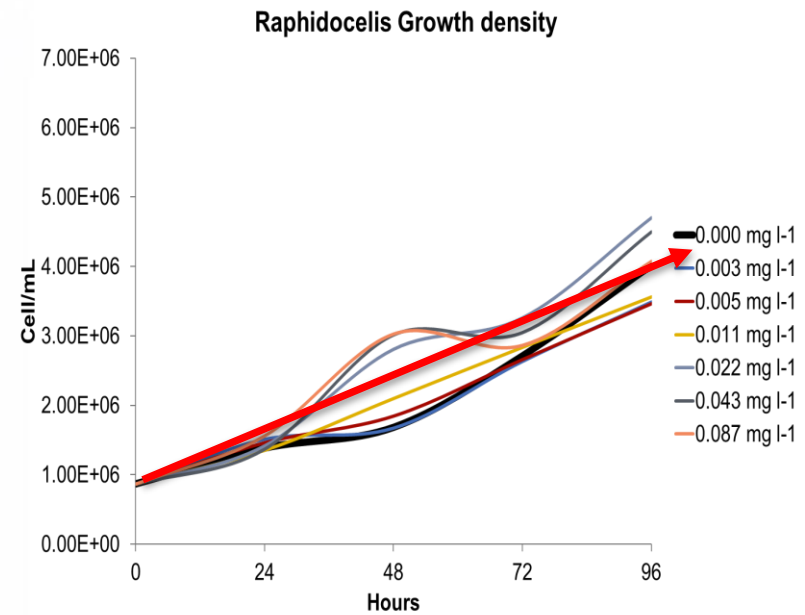
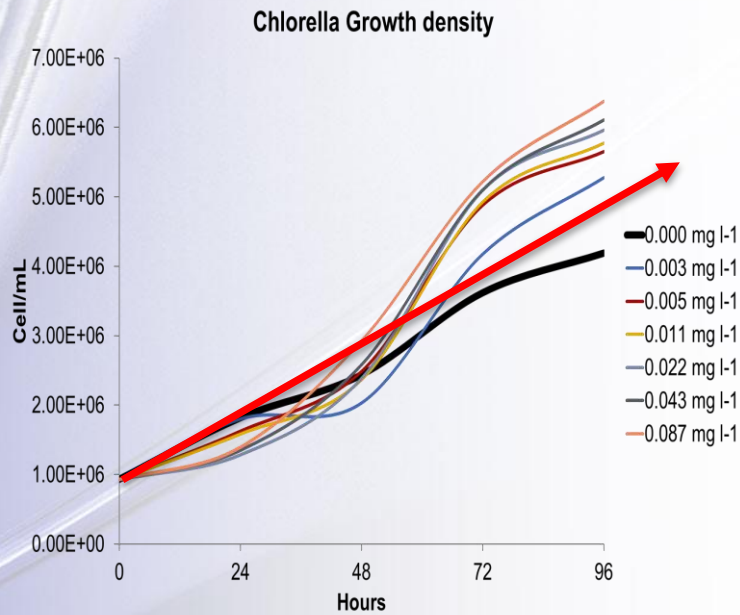
Chlorella Index Effect



Raphidocelis Index Effect

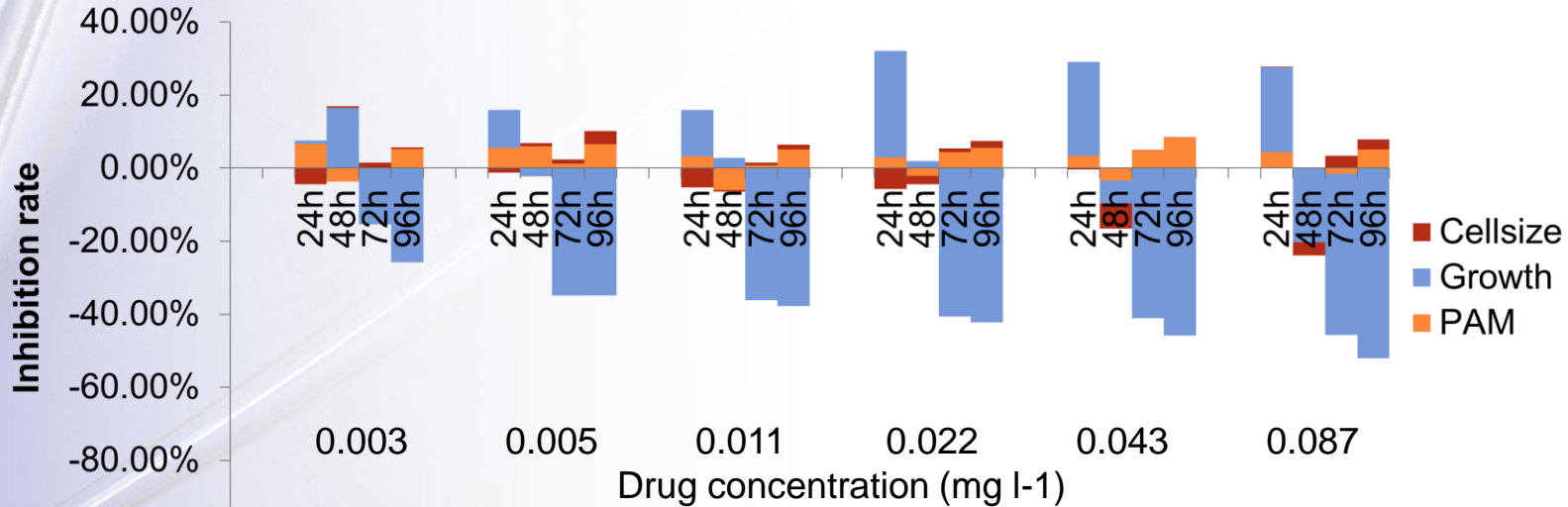


Results: Atenolol effects on algae culture

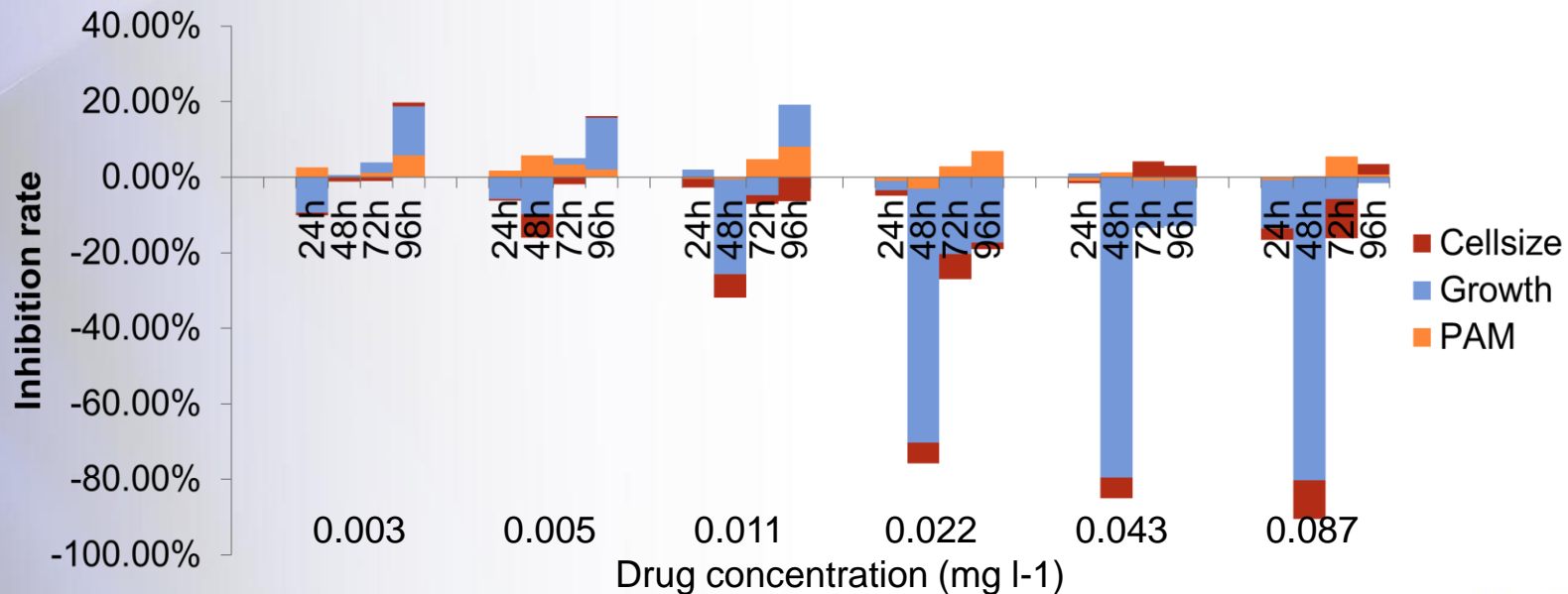


Results: Atenolol effects on algae culture

Chlorella PAM, Growth and Cell size analyze

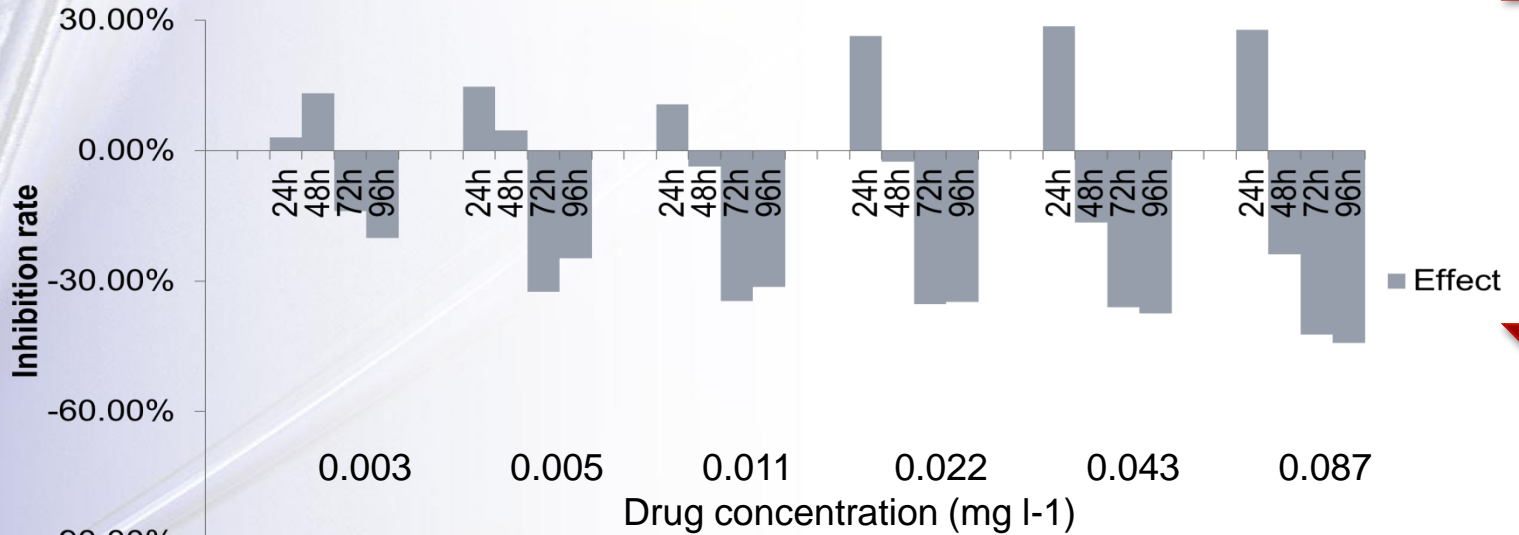


Raphidocelis PAM, Growth and Cell size analyze

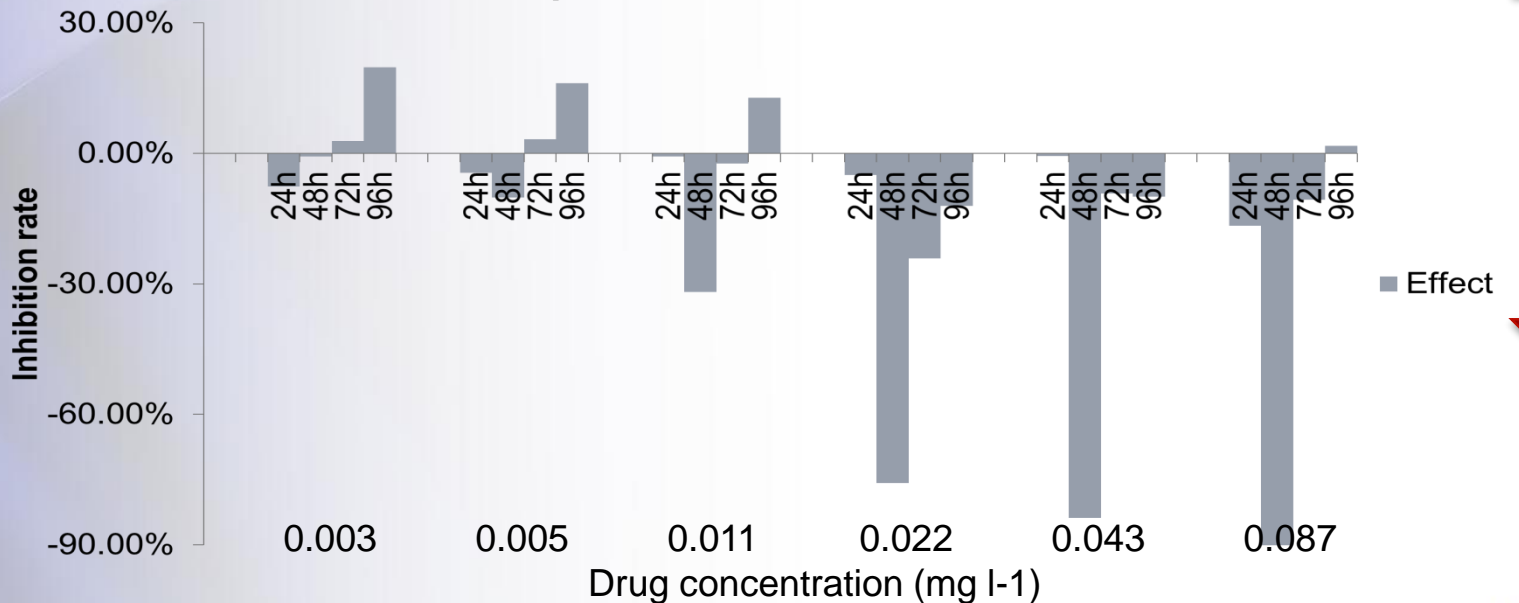


Results: Atenolol effects on algae culture

Chlorella Index Effect

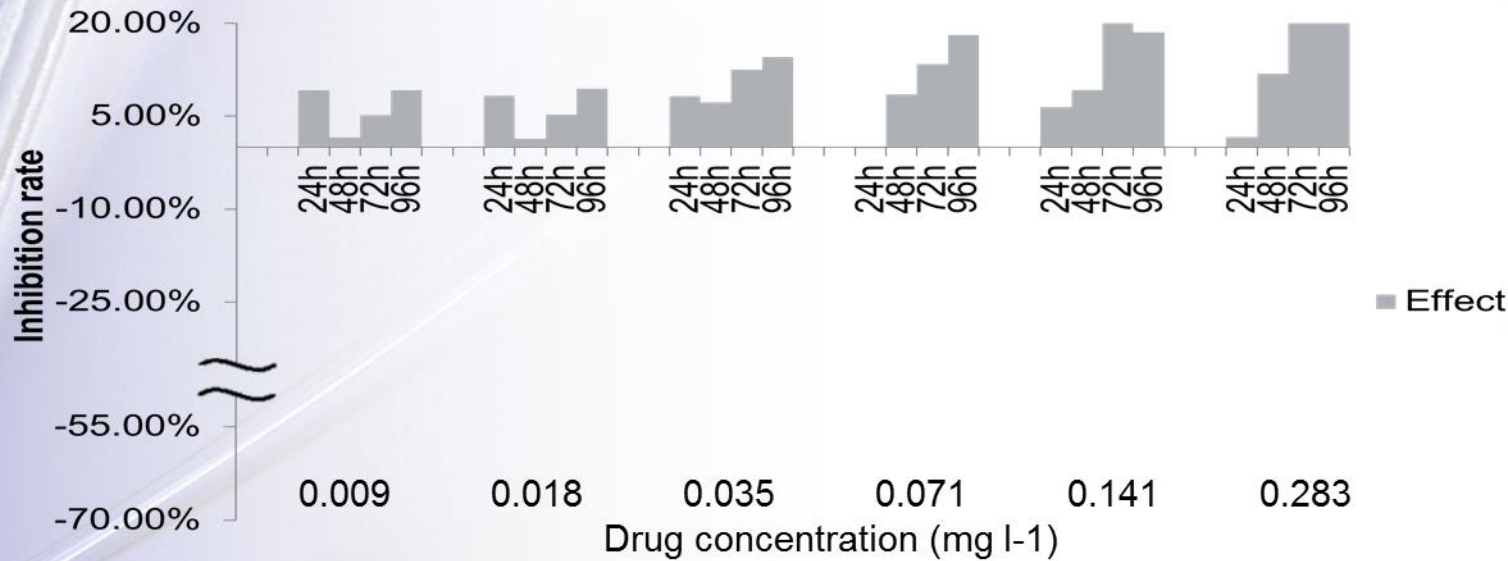


Raphidocelis Index Effect

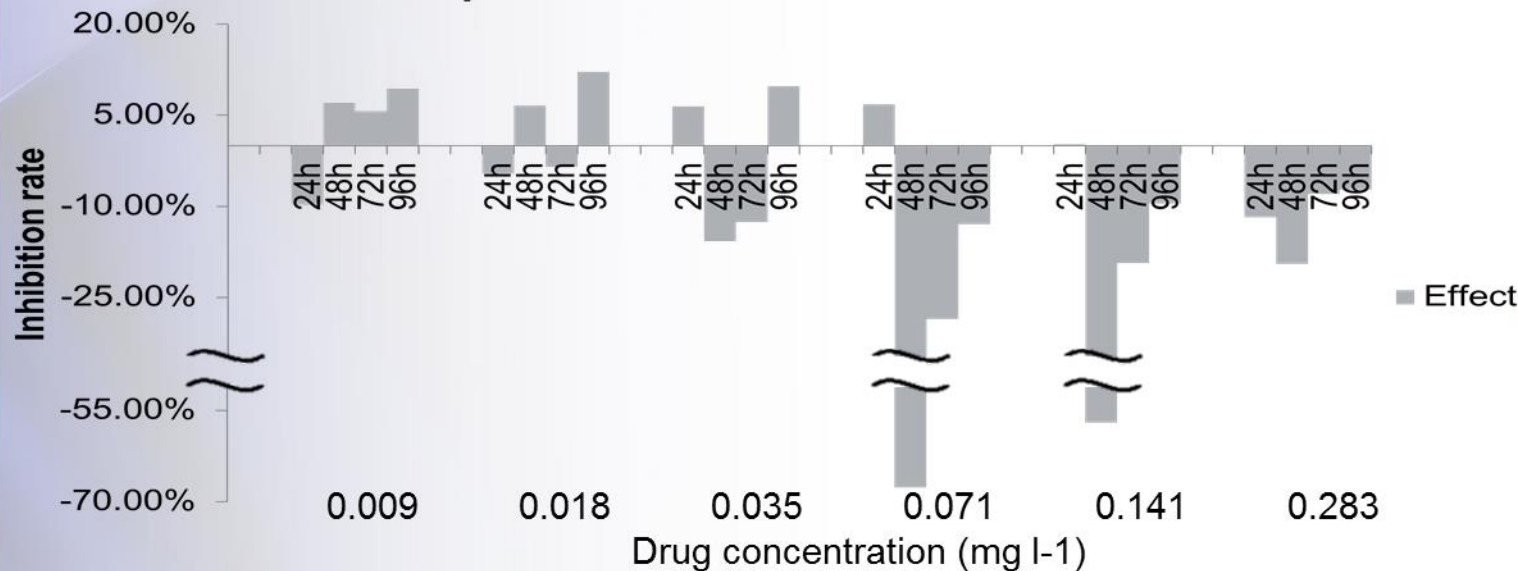


Results: Caffeine effects on algae culture

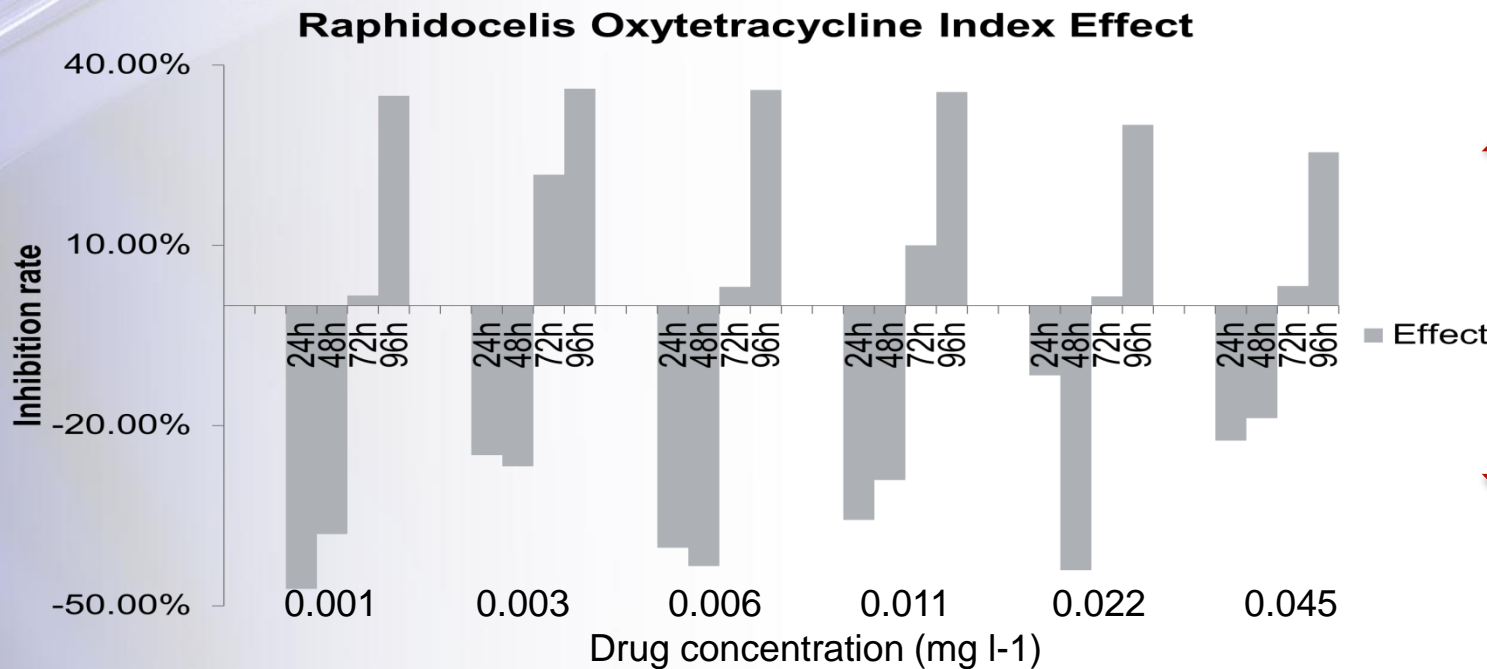
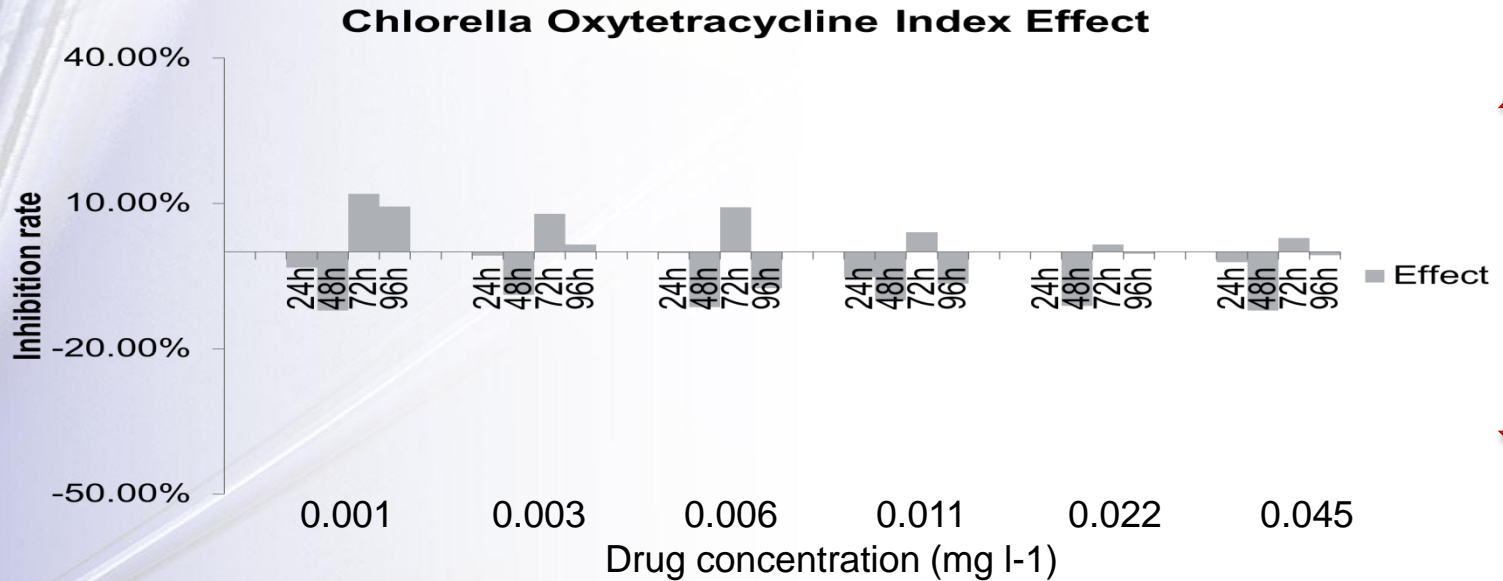
Chlorella Caffeine Index Effect



Raphidocelis Caffeine Index Effect



Results: Oxytetracycline effects on algae culture



Summary

- Atenolol:
 - *Chlorella*: Demonstrated a weak inhibition effect in the first 24h; nonetheless, in the end of the experiment, it presented a stimulation effect of 40%.
 - *Raphidocelis*: Strong stimulus at 48h, but not lasting until the end.
- Lidocaine:
 - *Chlorella*: Displayed significant inhibition of 40% in the beginning of the test; however, after 96h, the organism was in the same condition when compared to the control.
 - *Raphidocelis*: Crescent inhibition effect finishing at 50% after 96h.
- Caffeine:
 - *Chlorella*: No expressive effect with less than 20% of inhibition in the index effect.
 - *Raphidocelis*: Same response as Atenolol.
- Oxytetracycline:
 - *Chlorella*: Did not present significant response to the presence of the drug.
 - *Raphidocelis*: Significant stimulus in the first 24h, however after 96h the predominant effect was an inhibition with around 30% when compared to the control.



Conclusions

- *Chlorella* presented diverse behaviours according the drugs tested. In some cases, it suffers an initial inhibitory effect, although it did not last until the end of the experiment; in other conditions, this algae did not undergo to inhibitory/growth effects.
- *Raphidocelis* was more affected by the drugs tested, when compared to *Chlorella*. These results show how different organisms, with different morphologies, behave when dealing with the stress caused by presence of potential toxic compounds.
- The index effect support the appreciation of diverse mechanisms associated to the presence of drug within algae populations and the direct consequences on growth, cell size and photosynthetic activity.



Acknowledgment

