Effect of Benzalkonium Chloride on Chlorophyll Fluorescence and Growth of Freshwater Phytoplankton Species

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Introduction:

Benzalkonium Chloride (BAC) is a major compound used as a preservative in disinfectants, antimicrobials, and surfactants. BAC regularly enters the environment through wastewater and has been proven to be toxic to bacteria, crustaceans, fish, and invertebrate due to its biocide properties (Lavorgna et al., 2015). Moreover, low concentrations of BAC are used as an algicide to control algal growth in freshwater aquaculture and agriculture ponds, thus has negative effects on the growth of phytoplankton and other algae (Lee et al., 1994). BACs are not regulated yet since the EPA states that there is insufficient data in the literature (Pereira and Tagkopoulos, 2019). BAC has become a more significant threat to aquatic ecosystems since the first Corona outbreak in March 2020, due to a significant increase in the use of hand sanitizer and disinfectants (Mahmood et al., 2020).

Objective:

To determine the effect of relatively low BAC concentrations on the growth rate and the chlorophyll fluorescence of two common freshwater phytoplankton species, *Ankistrodesmus falcatus* and *Chlorella* sp.

Methods:

General Set-Up

- Cultures of *Ankistrodesmus falcatus* and *Chlorella* sp. were grown in a light- and temperature-controlled incubator
- Exposed to BAC concentrations of 0, 50, 100, 200, 400 µg/L
- BAC added after sampling on day 2
- Triplicate experimental treatments

Chlorophyll Fluorescence

- *In vivo* chlorophyll fluorescence monitored daily for 8 days (Turner fluorometer)

Cell abundance

- After fluorescence readings, samples were preserved in 5% Acid Lugol's
- Cells counted in Palmer-Maloney or Sedgewick-Rafter chambers (x400)

Statistics

- General Linear Models with Bonferroni post-hoc comparisons (SPSS)

Ankistrodesmus falcatus | ***Chlorella* sp.**

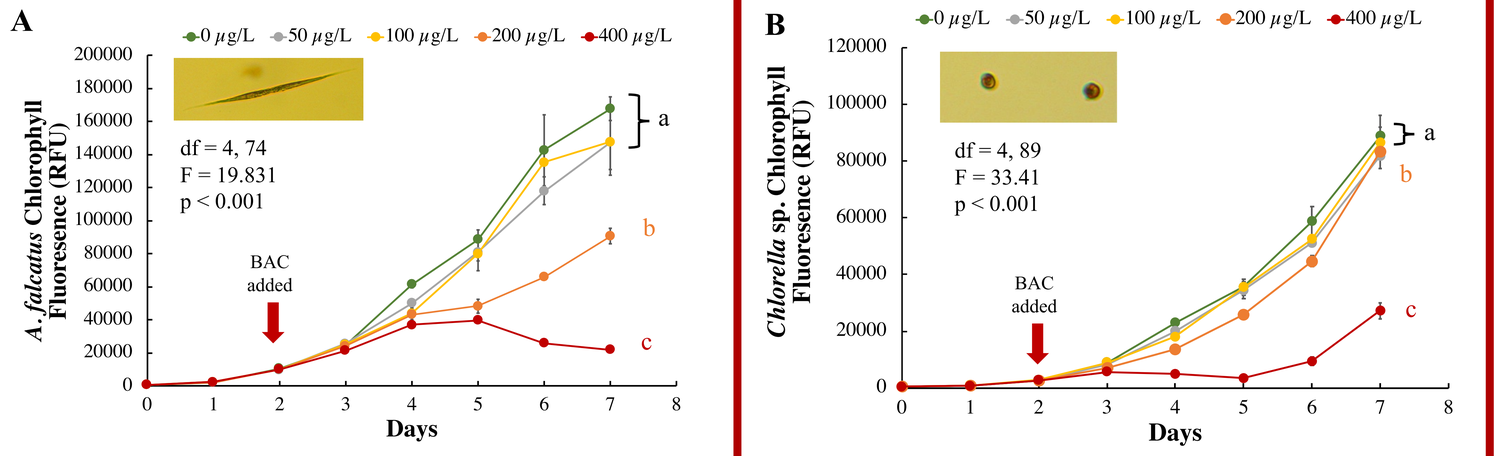

Figure 1. *A. falcatus* **(A)** and *Chlorella* sp. **(B)** chlorophyll fluorescence in relative fluorescent units (mean ± 1 SE) at BAC concentrations of 0 - 400 µg/L from day 0 to 7. BAC added after day 2. Different letters indicate significant differences between BAC treatments over time.

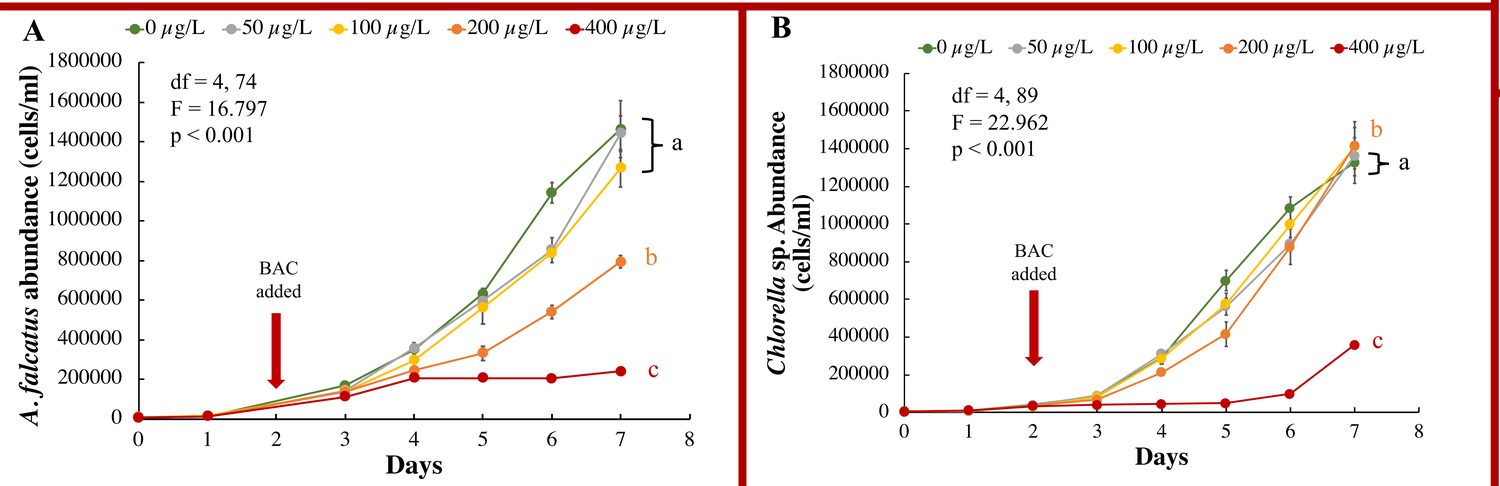

Figure 2. *A. falcatus* **(A)** and *Chlorella* sp. **(B)** cell abundance (mean ± 1 SE) at BAC concentrations of 0 - 400 µg/L from day 0 to day 7. BAC added after day 2. Different letters indicate significant differences between BAC treatments over time.

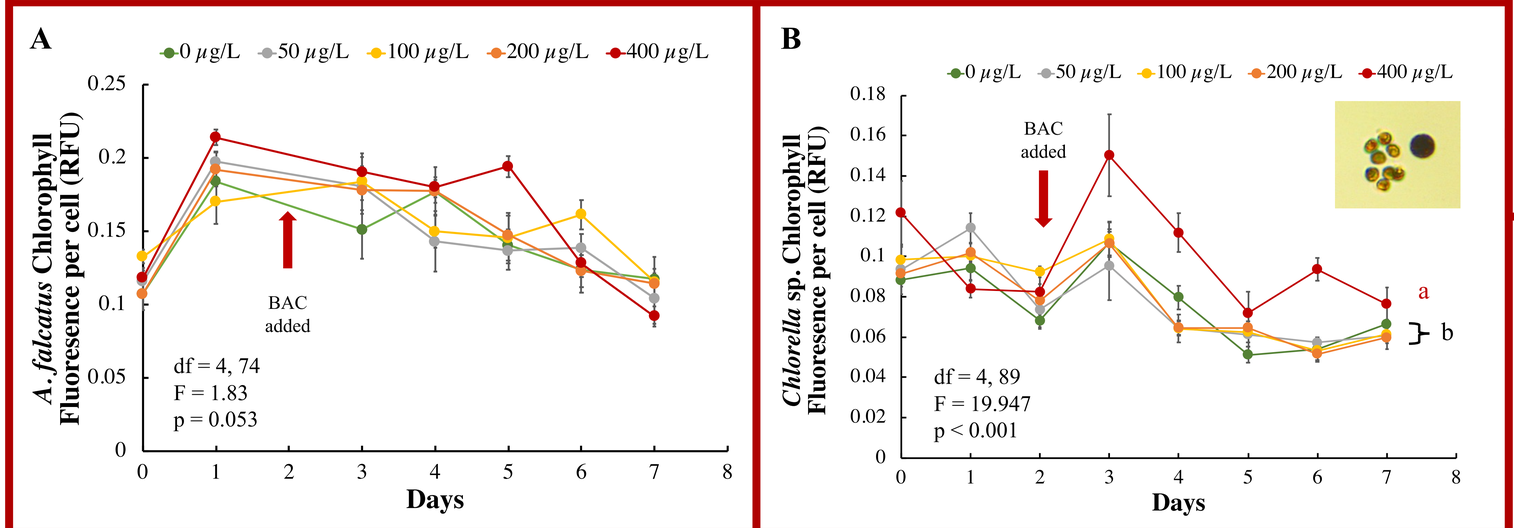

Figure 3. *A. falcatus* **(A)** and *Chlorella* sp. **(B)** chlorophyll fluorescence per cell (mean ± 1 SE) at BAC concentrations of 0 - 400 µg/L from day 0 to day 7. BAC added after day 2. Different letters indicate significant differences between BAC treatments over time.

Results

Fluorescence and cell abundance:

- *A. falcatus*: BAC concentrations of 200 and 400 µg/L significantly limited relative fluorescence and cell growth rates
- *Chlorella* sp.: BAC concentrations of 400 µg/L significantly limited relative fluorescence and cell growth rates
- Both species: no significant differences between BAC concentrations of ≤ 100 µg/L and control (0 µg/L)
- Both species showed some recovery after day 5 or 6: *A. falcatus* at 200 µg/L but not at 400 µg/L; *Chlorella* sp. At 400 µg/L

Fluorescence per cell :

- *A. falcatus*: RFU/cell not significantly affected by the BAC concentrations used
- *Chlorella* sp.: RFU/cell was higher in 400 µg/L BAC treatment

Discussion

- Relatively low BAC concentrations (200 µg/L or higher) can limit the growth of two freshwater phytoplankton species
- Impact of BAC takes time (> 24 hours), and cultures are able to recover if given enough time and the initial BAC concentration was not too high
- *A. falcatus*: BAC does not affect chlorophyll fluorescence inside individual cells, indicating that the decrease in cell growth is not due to damage to the light-harvesting apparatus of the cell
- *Chlorella* sp.: high BAC concentrations lead to higher fluorescence per cell, probably due to larger cell size at higher BAC concentrations
- Other sub-lethal effects should be investigated (effect on cell size & cell division)

References:

Lavorgna, M., Russo, C., D'Abrosca, B., Parrella, A., & Isidori, M. (2016). Toxicity and genotoxicity of the quaternary ammonium compound benzalkonium chloride (BAC) using *Daphnia magna* and *Ceriodaphnia dubia* as model systems. *Environmental Pollution*, *210*, 34-39.

Lee, C. L., Chen, I. M., & Fong, S. C. (1994). Effects of benzalkonium chloride on growth of phytoplankton. *臺灣水產學會刊*, *21*(1), 69-77.

Mahmood, A., Eqan, M., Pervez, S., Alghamdi, H. A., Tabinda, A. B., Yasar, A., ... & Pugazhendhi, A. (2020). COVID-19 and frequent use of hand sanitizers; human health and environmental hazards by exposure pathways. *Science of the Total Environment*, *742*, 140561.

Merchel Piovesan Pereira, B. & Tagkopoulos, I. (2019). Benzalkonium chlorides: uses, regulatory status, and microbial resistance. *Applied and Environmental Microbiology* 85.13: e00377-19.