COMPARISON OF FOUR METHODS TO ESTIMATE ALGAL BIOMASS IN PESTICIDE TOXICITY ASSAYS

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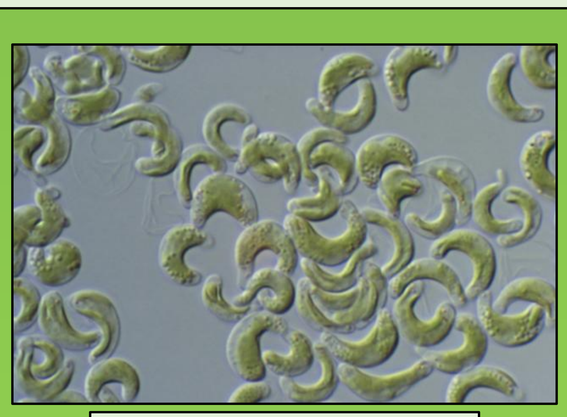
Introduction

The application of pesticides is an essential step in modern, agrochemicals-based agricultural technologies, while pesticide residues can enter into surface waters due to run-off, drifting and leaching of the chemicals. The mode of action and efficiency of numerous herbicides is based on the inhibition of photosynthesis, making them highly hazardous to photoautotrophic algae. Freshwater microalgae (e.g., *Pseudokirchneriella subcapitata*) are indicator organisms for environmental risk assessment. Evaluation of algal cell density and biomass upon exposure is necessary for the evaluation of the inhibitory potency and to calculate corresponding EC_{50} values on algal growth.



Pseudokirchneriella subcapitata

Materials and methods

The algal biomass of the undiluted batch culture of *P. subcapitata* and its dilution series (3x, 9x, 27x, 81x) were determined by counting individual algal cells in a Bürker counting chamber using inverted microscope, and algal viability was expressed by the use of additional surrogate parameters including optical density, chlorophyll-a (Chl-a) content and fluorescence. Optical densities were measured at 750 nm spectrophotometrically. Laser-induced Chl-a fluorescence (maximal level of fluorescence emitted by cells after dark adaptation (Fp)) of algal cells was detected using two different portable instruments (FluoroMeter Modul (FMM Fluoromodul System) and Chlorophyll Fluorometer (CFM)) at wavelengths 690 nm and 735 nm [1], while Chl-a content was determined from the samples using the corresponding standardised protocol [2]. The selected methods were applied for the evaluation of phytotoxic effects of a glyphosate-based formulation and its components on *P. subcapitata* as well [3].

Dilution series of *Pseudokirchneriella subcapitata*

CFM Chlorophyll Fluorometer

FMM Fluoromodul System

Results

The coefficient of determination for different methods applied for estimation of algal biomass and for evaluation of growth inhibition were statistically calculated. Correlation coefficients (R^2) between biomass results determined in a Bürker counting chamber and by optical density were 0.999±0.001 in three independent experiments (*Figure 1*). R^2 values between Chl-a content and Chl-a fluorescence were 0.997±0.003 and 0.997±0.002, respectively, however, for CFM intensitiy of the fluorescent analytical signal was approximately 100 times higher than for FMM (*Figures 2, 3*). The effects of the herbicide active ingredient glyhposate, along with its formulated herbicide (Roundup Classic) and co-formulant (polyethoxylated tallowamine, POEA) on the biomass and Chl-a content of *P. subcapitata* were also determined. At low (< 20 mg/l) glyphosate concentrations, an increase in biomass and Chl-a content were determined (hormesis), indicating that glyphosate may serve as a nutrient phosphate source for algae [4]. At higher concentrations, however, phytotoxicity prevailed over the hormesis effect observed. POEA exerted substantial phytotoxicity (> 2 mg/l) both in neat and formulated forms.

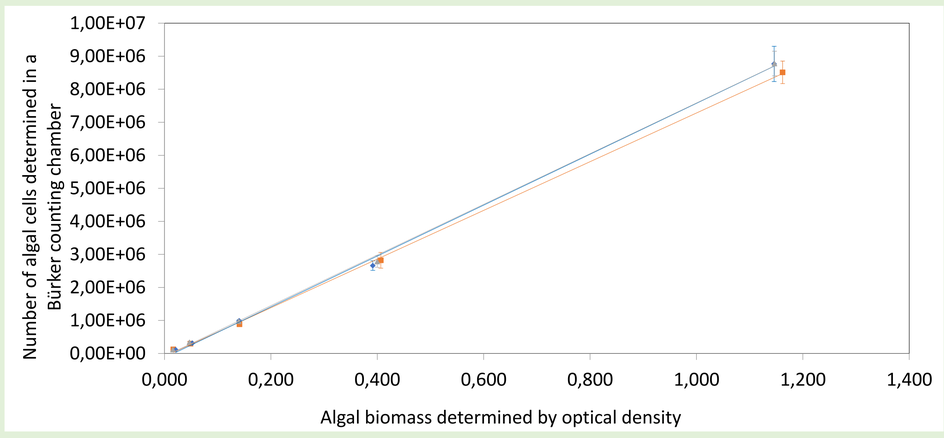


Figure 1. **Correlation between Bürker counting and optical density in determination of algal biomass**

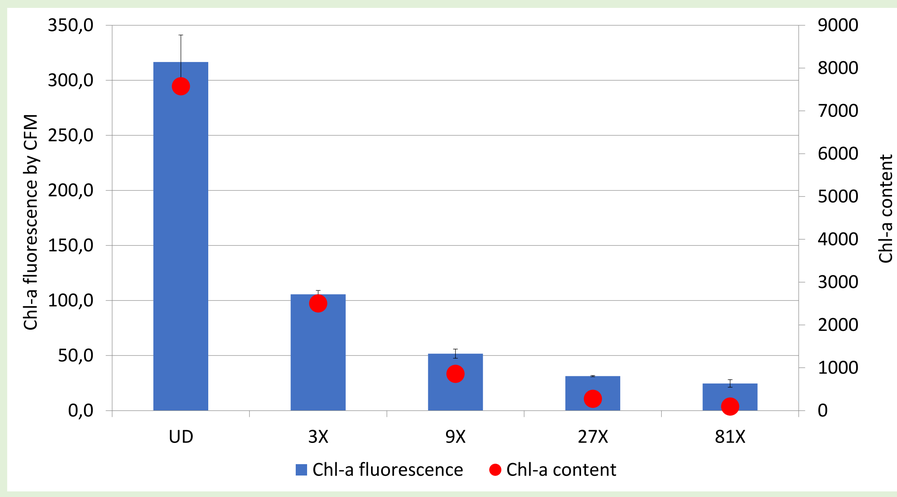


Figure 2. **Correlation between Chl-a fluorescence determined by CFM and Chl-a content determined by OECD guideline at different dilution rates of *P. subcapitata* (UD=undiluted)**

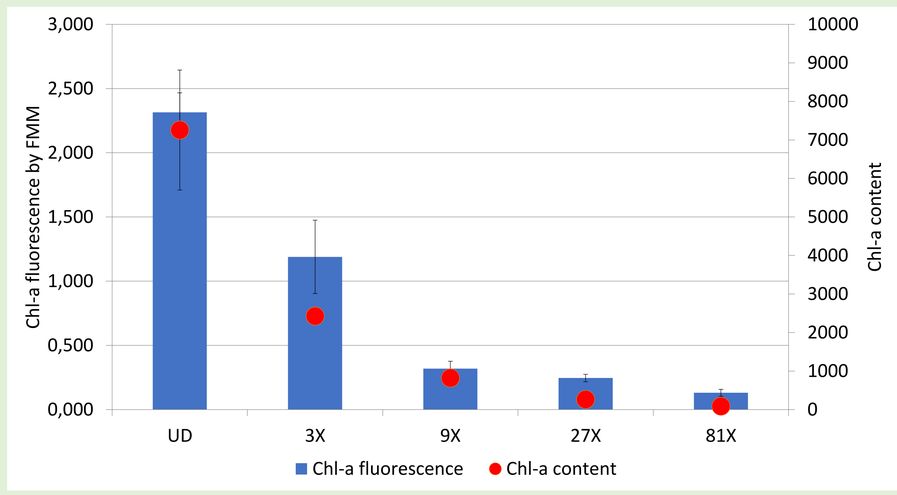


Figure 3. **Correlation between Chl-a fluorescence determined by FMM and Chl-a content determined by OECD guideline at different dilution rates of *P. subcapitata* (UD=undiluted)**

References

[1] Barócsi, A.; Kocsány, L.; Várkonyi, S.; Richter, P.; Csintalan, Z.; Szente, K. *Meas. Sci. Technol*. **2000**, *11*, 717-729.
[2] OECD *Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test*; OECD Publishing: Paris, 2011; p. 25.
[3] ISO 10260:1992; International Organization for Standardization: Geneva, 1992; p. 6.
[4] Klátyik, Sz.; Takács, E. ; Mörtl, M. ; Földi, A. ; Trábert, Zs. ; Ács, É. ; Darvas, B. ; Székács, A. *Int. J. Envir. Anal. Chem.* **2017**, *97*,901-921.

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