

Risk assessment of chemicals: a pH-measuring system to monitor the effects of pollutants on aquatic macrophytes



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Introduction

In toxicity tests with macrophytes the usual endpoints are mostly based on growth parameters. Monitoring physiological parameters in addition to growth can improve the sensitivity and the informative value of single species tests with macrophytes. Photosynthetic activity would be a suitable endpoint because of its fundamental relevance for plants.

To quantify the photosynthetic activity, the increase of the pH, due to CO₂ consumption by photosynthesis, in the medium surrounding the aquatic plants was continuously measured in single species tests. For this purpose a highly-sensitive and computer-controlled pH-measuring system was developed, which allows a continuous monitoring of very small pH-changes. The diurnal pH-changes of the medium give information about photoinhibition and the kinetic of toxic effects.

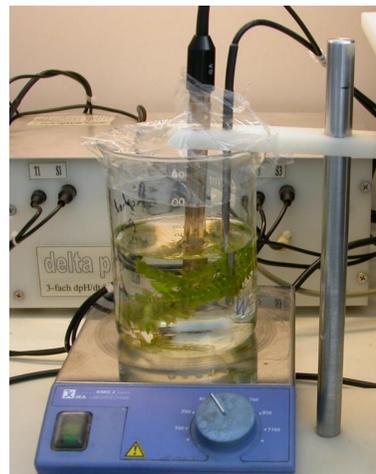
With this method toxic effects on *Elodea canadensis* of the cationic detergent benzalkonium chloride and the photosynthesis inhibiting herbicide atrazine could be observed and quantified within hours.

Materials & Methods

An automated and highly sensitive pH-measurement system with multiple pH-electrodes was developed (Dr. V. Gerhardt, Faculty of Physics, Regensburg, Germany). Changes of 1/10000 pH-units can be registered. Each electrode of the measurement system was dipped in a 600 ml beaker filled with 400 ml Medium.

The measurements were performed in a large closed box with integrated lightening.

- **Test plant:** *Elodea canadensis*
- **Test medium:** 20X-AAP growth medium (OECD guideline 221) diluted 1:1 (v/v) with aqua dest.
- **Light / dark rhythm:** 16 h / 8 h



First outcome

- For atrazine EC₅₀ of growth can be reproduced by online-pH-Measurements in the medium
- Depending on the mode of action of the test item effects can be monitored within 24 h
- Online measurements of pH allow recording the kinetic of the effect
- Non-invasive method
- Can be easily adapted to various species of aquatic macrophytes (e.g. *Ceratophyllum demersum*, *Elodea canadensis*, *Riccia fluitans*)

Results

In fig 1 toxic effects of the detergent Benzalkonium chloride on the photosynthetic activity of *Elodea canadensis* are presented.

In curve A (control) during all the experiment in each light phase an increase of the pH of the medium due to the photosynthesis of the plants can be recognized. During dark phases a decrease of pH due to CO₂ production by respiration was observed. In curve B the detergent inhibited in less than 2 hours after application (1 mg/l) the photosynthetic activity of *Elodea*. No further increase of the pH was measured. 4 hours after application of the detergent a decrease of the pH was observed. Photosynthesis was completely inhibited but respiration was still working. Benzalkonium chloride interacts with biological membranes and induces damages in chloroplast membranes and so impairs photosynthesis. In fig 2 the effect of different concentrations of atrazine on the photosynthesis of *Elodea canadensis* is represented. After atrazine application concentration dependant decreases in photosynthetic activity could be observed during the following light phase. At 50 µg/l an inhibition of photosynthesis of 75% was measured. At 200 µg/l no pH-increase was observed but a pH decrease which means that photosynthesis was completely inhibited and CO₂ was released by respiration. Similar values obtained with other test methods are found in literature. In a 14 days growth inhibition test with *Elodea canadensis* an EC₅₀ value of 21 µg/l was determined [1].

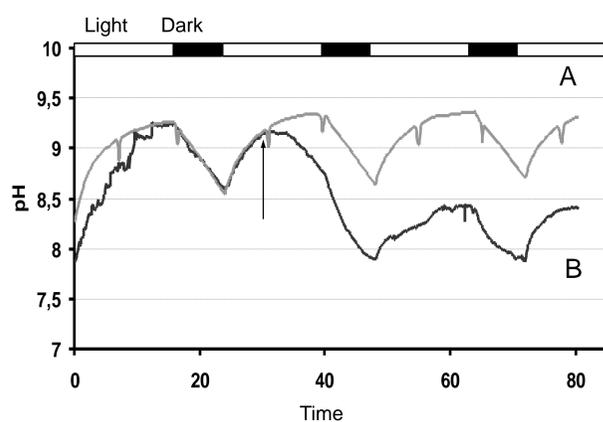


Fig. 1: Effect of Benzalkonium chloride (1 mg/l) on the photosynthetic activity of *Elodea canadensis*. Curve A: Control untreated plant. Curve B: Time of the application: 30th hour (arrow). Light / dark 16 h / 8 h.

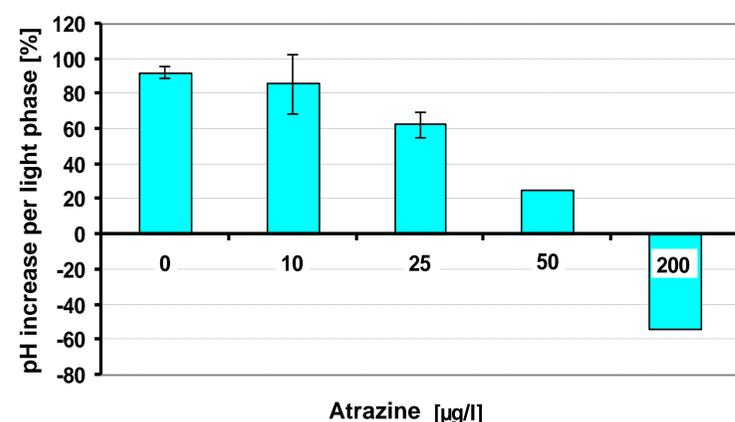


Fig. 2: Effect of atrazine on the photosynthetic activity of *Elodea canadensis* measured by pH changes. Atrazine was applied at the beginning of a light phase and the pH increase during this 16 h light phase is plotted. The pH increase during the light phase preceding the application of atrazine was used as reference and set as 100%. n=3.