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IN-SITU BIOASSAY WITH MACROPHYTES IN MESOCOSM POND STUDIES

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Introduction

In higher-tier ecotoxicological testing of plant protection products, outdoor mesocosm ponds serve as models for aquatic ecosystems. Guidance documents recommend adding macrophytes to the ponds since they are the key species for herbicide testing, and they are also thought to increase the diversity of the biocoenosis in the model systems.

However, macrophyte growth and species composition are difficult to control in mesocosm ponds. This often leads to high variability between and within treatment doses. Due to this low reproducibility, quantification of toxic effects on water plants in mesocosms is usually very difficult. Moreover, the ponds are often dominated by few or only one fast growing macrophyte species.

For the above-mentioned reasons, an in-situ bioassay with water plants was developed and tested for its utility in mesocosm ponds.

Materials and Methods

Elodea canadensis, *Myriophyllum spicatum*, *Potamogeton lucens*, and *Chara globularis* were planted evenly spread into plastic pots (40 x 16 cm) with natural sediment from the mesocosms. For the emerse species *Glyceria maxima*, an equal number of sprouts were planted into plastic pots with a surface area of 60 x 16 cm. All macrophyte pots were inserted into outdoor mesocosm ponds (diameter of 2.5 m, water depth approx. 1.0 m, volume of water approx. 4900 litres) in early May. One pot per species and pond was incubated on trays, fixed at a water depth of about 0.3-0.4 m at the pond walls.

In the case of *Elodea canadensis*, *Myriophyllum spicatum*, *Potamogeton lucens*, and *Chara globularis* the mean length of the main plant shoots and the water surface area covered by the plants were regularly quantified. For *Glyceria maxima* the increase in the number of sprouts was counted instead of quantifying of the surface coverage area. The plants were harvested for biomass determination (wet and dry weights) 6 – 8 weeks after the test substance application.

Minimum detectable difference (MDD):
The MDD indicates the lowest difference between control and treatment, which could be detected as significant by a statistical test. The calculation based on the Student t-test, for 4 controls and 2 treatment replicates, one-sided with a 5% level of significance. The %MDD represents the relative minimum detectable difference between control and treatment.

$$MDD = \bar{x}_1 - \bar{x}_2 = t^* \cdot \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} \qquad \%MDD = 100 \cdot MDD / \bar{x}_1$$

$\bar{x}_1, \bar{x}_2$: arithmetic mean of a variable of control and treatment
s_1^2, s_2^2: standard deviation of a variable of control and treatment
n_1, n_2: number of replicates in control and treatment
t*: critical t value for $\alpha = 0.05$, degree of freedom = n_1+n_2-2

Results and Discussion

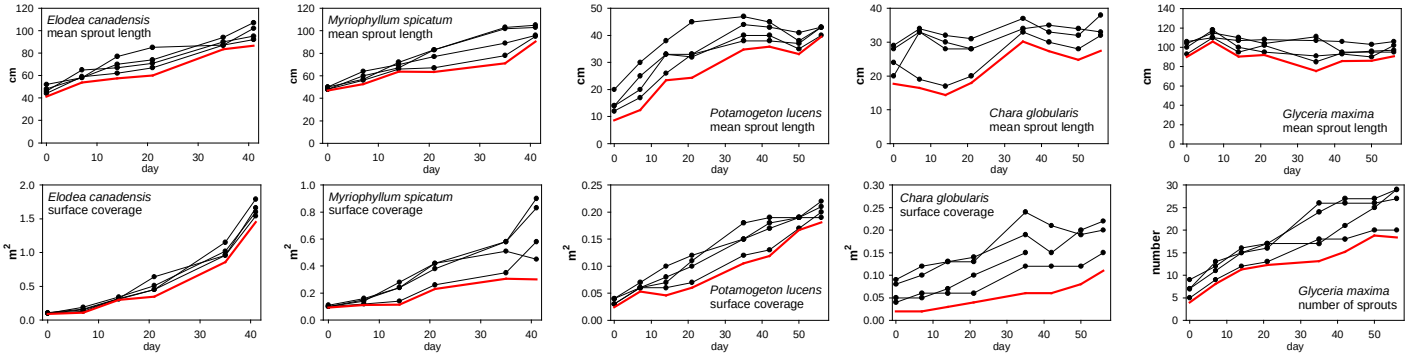

Fig. 1: Growth of the water plants in four control ponds (**black lines**). Values below the **red line** (mean of controls – MDD) would be significant different to the controls.

Table 1: Growth of the water plants of the in-situ bioassay in four parallel control ponds. Classes for %MDD: ++ (<10%), +(<20%), - (>20%), -- (>50%)

Taxon	Endpoint	Day	Mean ± SD	% MDD	
Elodea canadensis	mean sprout length	41	99.0 ± 6.8 cm	13	+
Elodea canadensis	surface coverage	41	1.65 ± 0.11 m²	12	+
Elodea canadensis	dry weight	42	179.1 ± 6.0 g	7	++
Myriophyllum spicatum	mean sprout length	41	99.8 ± 5.0 cm	9	++
Myriophyllum spicatum	surface coverage	41	0.69 ± 0.21 m²	56	--
Myriophyllum spicatum	dry weight	42	64.8 ± 26.0 g	86	--
Potamogeton lucens	mean sprout length	56	42.3 ± 1.5 cm	7	++
Potamogeton lucens	surface coverage	56	0.21 ± 0.01 m²	12	+
Potamogeton lucens	dry weight	56	21.3 ± 2.9 g	29	-
Chara globularis	mean sprout length	56	34.2 ± 3.2 cm	18	+
Chara globularis	surface coverage	56	0.19 ± 0.04 m²	37	-
Chara globularis	dry weight	56	59.2 ± 29.5 g	107	--
Glyceria maxima	mean sprout length	56	100.0 ± 5.0 cm	9	++
Glyceria maxima	number of sprouts	56	26.3 ± 4.3	30	-
Glyceria maxima	dry weight	56	60.4 ± 3.3 g	12	+

All selected water plant species are suitable in practice. The methods of growth evaluation should however be species specific. At least one of the three endpoints always showed a %MDD below 20% at each plant species (Tab. 1), indicating that this endpoint is well applicable for the determination of toxic effects of the test substance on the macrophyte growth.

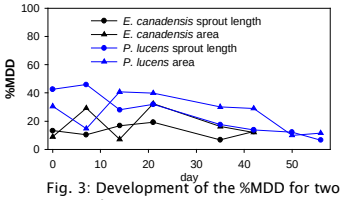

Fig. 3: Development of the %MDD for two macrophyte species over time.

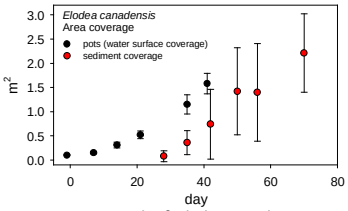

Fig. 4: Growth of *Elodea canadensis* in pots and of non-planted sprouts in the sediment on the pond bottom (mean ± SD).

The %MDD for the acceptable endpoints often decreased with time (Fig. 3). Thus, the test period for the water plants should be at least 4 – 8 weeks after the test substance application.

Elodea canadensis plants started to grow also in the sediment at the bottom of the ponds about four weeks delayed. These plants showed a similar growth rate compared to the potted plants. However, the variability in growth of the potted plants was much lower than in the uncontrolled plants on the sediment (Fig. 4).

Conclusions

The in-situ bioassay with potted plants shows several advantages compared to an uncontrolled growth of water plants in the sediment on the bottom of the ponds:

⇒ The exposure of the plants in pots near the water surface allows the regular short removal of the pots for observation and measurements of toxic effects on the plants during the exposure period. Qualitative effects such as colour changes can be easily recorded, but also shoot length or surface coverage can regularly be measured not only at the end of the study but already during the exposure period.

⇒ The variability in the growth of potted plants is less than in uncontrolled growth of water plants, developing on the natural sediment in the mesocosm ponds. Thus, the bioassay allows a more precise determination of toxic effects of a test substance on water plants.

⇒ In the bioassay several plant species can be tested in parallel, without overgrowth of a species by another, faster growing species.

⇒ Fast growing, dominating plant species (e.g. *Elodea canadensis*) can be easily harvested at an earlier stage of the study, without disturbing the other plant species.

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