

Establishment of a flow-through system for the macrophyte growth inhibition test with *M. spicatum* (OECD TG 239) including new endpoints on photosynthetic activity

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Motivation

- Testing of test rapidly degradable substances in a *M. spicatum* growth inhibition test in accordance with the OECD test guidelines 239 by using a flow-through system.

Aims of the study

- Miniaturize the standard test system according to OECD TG 239 (see Figure 1) [7].
- Find an appropriate flow-through setup with an adequate nutrient concentration to fulfill the requirements of OECD TG 239 [7].
- Establish a flow-through system by revealing stronger effects for the degradable pharmaceutical atorvastatin (AV).
- Test photosynthetic activity (endpoint Yield PSII) with IMAGING-PAM on being a new early non-invasive endpoint using the PSII inhibitor bentazone (BT).

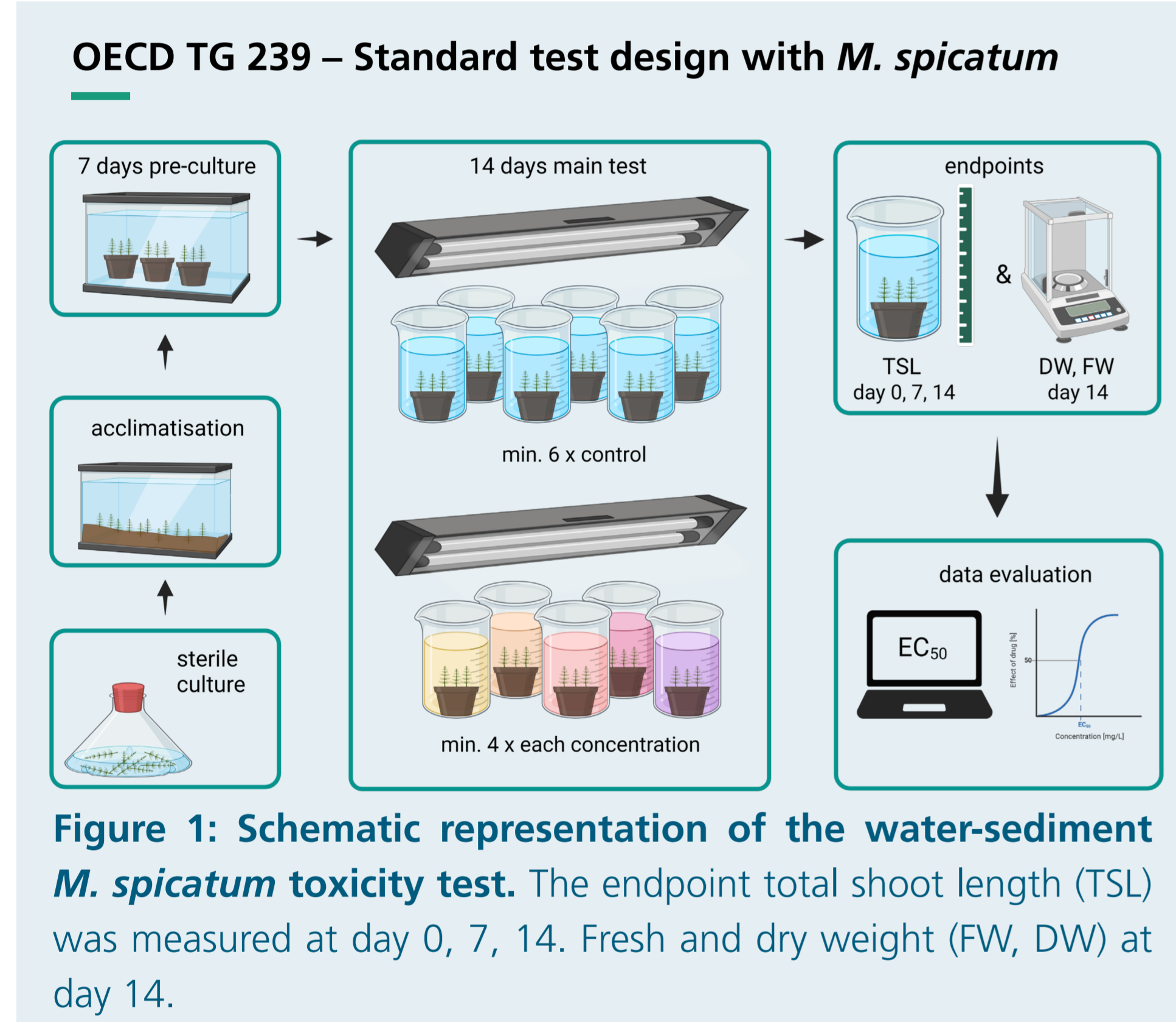


Figure 1: Schematic representation of the water-sediment *M. spicatum* toxicity test. The endpoint total shoot length (TSL) was measured at day 0, 7, 14. Fresh and dry weight (FW, DW) at day 14.

Results & discussion

Did the miniaturization succeed?

The linear regression using the growth rates revealed no differences between both setups. The EC₅₀ values were in line with the literature values [7].

→ Yes

Which nutrient concentration was appropriate for the flow-through setup?

Comparing a static control with one in flow-through obtaining the same nutrient concentration led to significant differences in height and weight. The static control against the flow-through with ¼ nutrient concentration revealed no difference for the total shoot length (TSL).

Was the flow-through system successfully established?

Comparing both setups with BT, the most sensitive endpoint resulted in EC₅₀ = 2.5 mgL⁻¹ corresponding to those found for *Lemna minor*. Both macrophytes were thus equally sensitive to the herbicide [3,6].

Table 1: Effects of the semi-static and flow-through test using atorvastatin.

	Semi-static		Flow-through	
	EC ₂₀	EC ₅₀	EC ₂₀	EC ₅₀
Growth rate fresh weight	4.36	n.d.	1.82	5.18
Growth rate total shoot length	1.64	n.d.	1.01	3.69
Growth inhibition	25%		44%	
Necrosis	in 3.00 mg/L from day 7		in 0.3 & 3.00 mg/L from day 7	

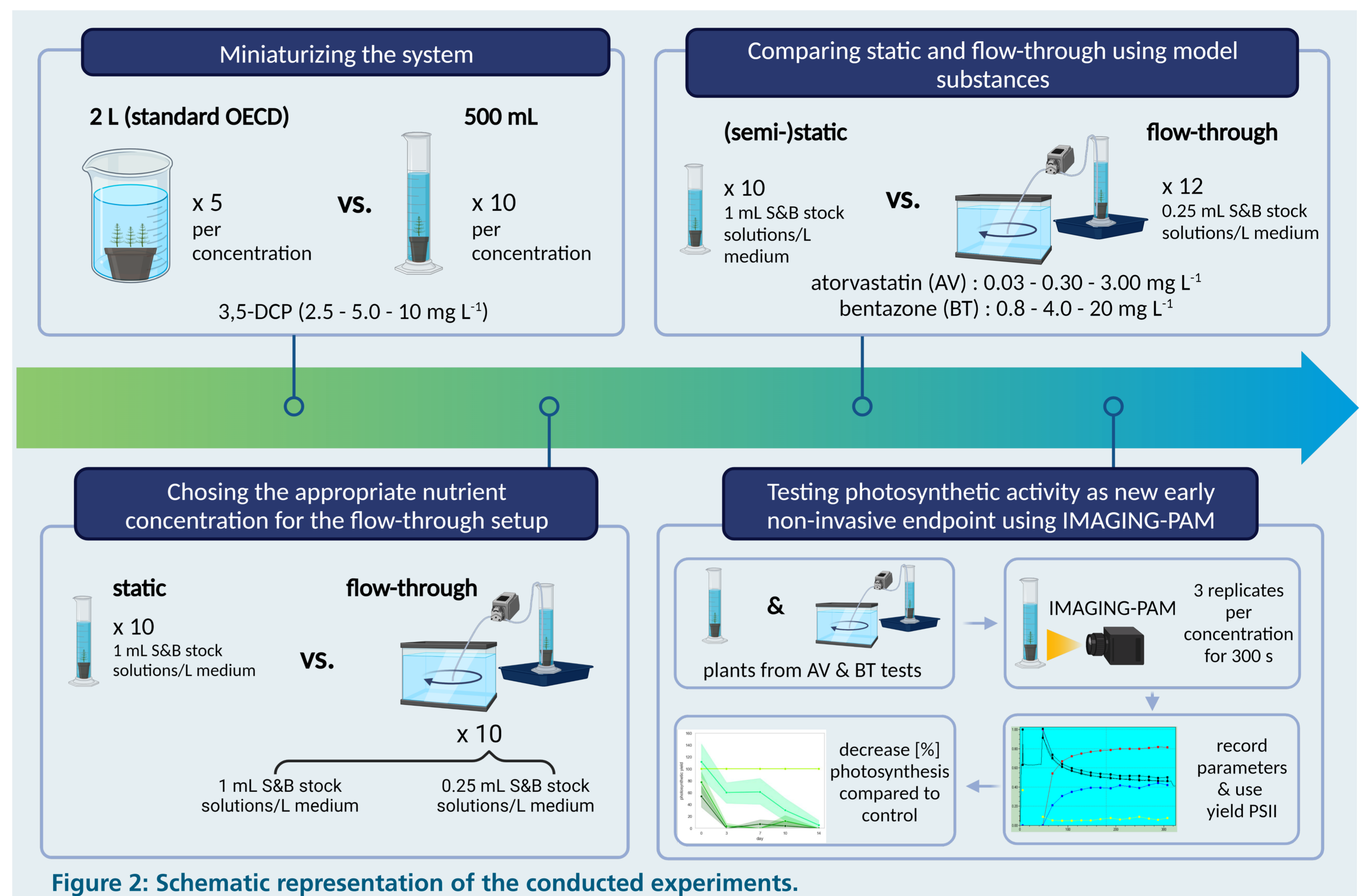


Figure 2: Schematic representation of the conducted experiments.

Atorvastatin degraded in the semi-static system by 35% after 7 days, whereas it remained stable using the flow-through setup. The degradation was traced back to photolysis [4] assuming a weaker effect in the semi-static system. The results reflected the hypothesis as shown in Table 1.

No literature using *M. spicatum* was available, resulting in a lack of information. Tests with *Lemna gibba* resulted in 15-times lower EC₅₀ values [1,2]. From this, Lemnaceae appear to be the more sensitive aquatic primary producer towards atorvastatin.

→ Yes

Is photosynthetic activity a promising new non-invasive early endpoint for the OECD TG 239?

Analyzing atorvastatin, no decrease in photosynthesis was observed.

For PSII inhibitor bentazone, a decrease in photosynthetic activity was already visible at day 3 for all concentrations (see Figure 3).

This method being more sensitive than conventional endpoints was also reported working with *M. aquaticum* and a marine diatom [5,8].

→ Yes

Conclusion & outlook

- Miniaturization & flow-through established.
- Idea of flow-through setup should be proceeded and, long-term, be established in the OECD TG 239.
- The Y(II) could serve as additional endpoint for knowing, whether the substance affects photosynthesis. An endpoint that can be measured during the test and does not interfere with the test system or disturb the growth.

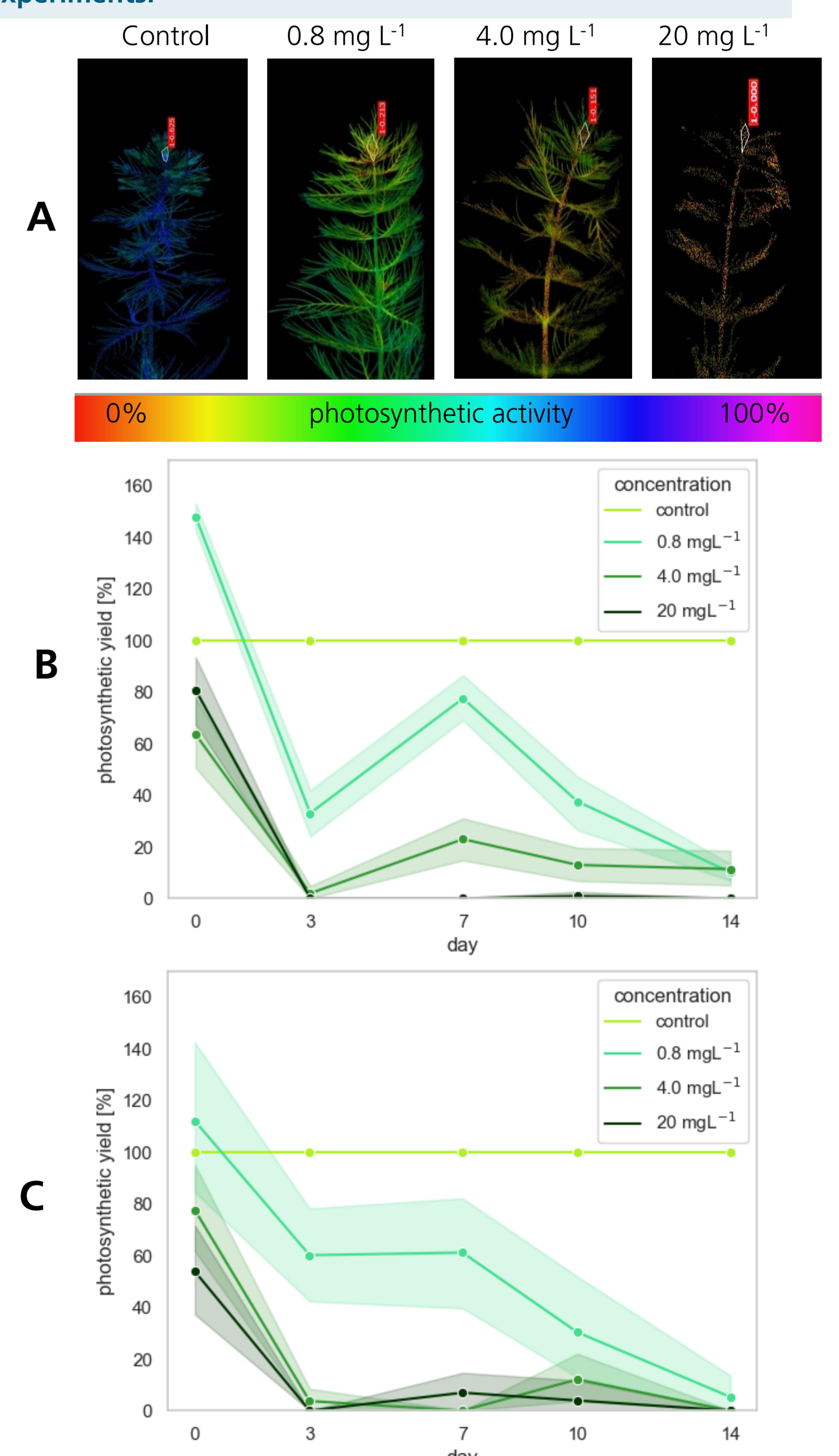


Figure 3: Photosynthetic activity (given as Y(II)) of bentazone treated plants. A: Y(II) over the test period of 2 weeks. **B:** plants from the flow-through test, **C:** plants from the static approach. At each time point, three plants per concentration were screened with the IMAGING-PAM.

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