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Sensitivity of submersed freshwater macrophytes and endpoints in laboratory toxicity tests

Gertie H.P. Arts [a,*], J. Dick M. Belgers [a], Conny H. Hoekzema [a], Jac T.N.M. Thissen [b]

[a] *Alterra, Wageningen University and Research Centre, Centre for Water and Climate, Droevendaalsesteeg 3, P.O. Box 47, 6700 AA Wageningen, The Netherlands*
[b] *Biometris, Wageningen University and Research Centre, The Netherlands*

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A range of endpoints is more representative of macrophyte fitness than biomass and growth only.

Abstract

The toxicological sensitivity and variability of a range of macrophyte endpoints were statistically tested with data from chronic, non-axenic, macrophyte toxicity tests. Five submersed freshwater macrophytes, four pesticides/biocides and 13 endpoints were included in the statistical analyses. Root endpoints, reflecting root growth, were most sensitive in the toxicity tests, while endpoints relating to biomass, growth and shoot length were less sensitive. The endpoints with the lowest coefficients of variation were not necessarily the endpoints, which were toxicologically most sensitive. Differences in sensitivity were in the range of 10–1000 for different macrophyte-specific endpoints. No macrophyte species was consistently the most sensitive. Criteria to select endpoints in macrophyte toxicity tests should include toxicological sensitivity, variance and ecological relevance. Hence, macrophyte toxicity tests should comprise an array of endpoints, including very sensitive endpoints like those relating to root growth.

Keywords: Submersed macrophytes; Laboratory tests; Endpoints; Variability; Sensitivity; Non-axenic

1. Introduction

Submersed macrophytes are an important functional and structural element of aquatic ecosystems, which fulfil several important functions in these systems (Scheffer, 1998). One of these functions is converting solar energy and carbon dioxide to organic matter, which in turn serves as a food resource and habitat and shelter for aquatic fauna (Fairchild et al., 1998). Submersed macrophytes are also important for oxygen production, nutrient cycling, controlling nutrient availability in the water layer and sediment stabilization (Lewis, 1995). They can thus be regarded as key species, and changes in the macrophyte community can have major consequences for the aquatic ecosystem.

Despite their important role in aquatic ecosystems, submersed macrophytes are only poorly addressed in the Environmental Risk Assessment procedures for pesticides and other toxicants. Tests with submersed macrophytes are only required for herbicides if risks to aquatic plants (as tested on *Lemna* spp.; OECD, 2002) have been identified. It is increasingly being questioned whether toxicity tests with *Lemna* can be regarded as representative of effects on macrophytes (Fletcher, 1990; Lewis, 1995; Hanson and Arts, 2007; Turgut and Fomin, 2001). Even if a test with *Lemna* is required, this could underestimate the toxicity to other, submersed macrophytes (Belgers et al., 2007; Cedergreen et al., 2004b; Lewis, 1995; Turgut and Fomin, 2002). For submersed macrophytes there is no internationally accepted guideline on laboratory or field tests. Although a guideline is available for Canada and North America (ASTM, 2004), this is not accepted as a standard by regulators and other stakeholders.

Lemna species have many advantages as test species. They can easily be cultured in the laboratory, in axenic as well as

* Corresponding author. Tel.: +31 317 478736; fax: +31 317 419000.
E-mail address: gertie.arts@wur.nl (G.H.P. Arts).

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non-axenic cultures. It is easy to achieve optimum growth conditions for *Lemna* in the laboratory and to establish exponential growth. Since growth rates are high (DeBusk et al., 1981), recovery is fast and recovery time is short. The short life cycle of *Lemna* species, however, is not representative of more slowly growing submersed macrophyte species (Cedergreen et al., 2005). In addition, *Lemna* leaves are exposed to air and water and can be directly influenced by pesticide drift onto their leaves. Submersed macrophytes, by contrast, are fully submersed in the water column, their leaves and stems being exposed to pesticide residues in the surface water, which enter the water body by spray drift, run-off, drainage or accidental spills. Moreover, submersed macrophyte roots may also be exposed via the sediment and pore water. *Lemna* species may, therefore, be more or less sensitive than submersed aquatic macrophytes, depending on species and compound (Belgers et al., 2007; Cedergreen et al., 2004a). These differences in life cycle, exposure routes and sensitivity make it uncertain whether test results obtained with *Lemna* species, and the application of an Assessment Factor of 10, can in all cases be protective for submersed aquatic macrophytes.

Tests with submersed aquatic macrophytes have to consider aquatic plant endpoints sensitive to the compound to be tested or to a series of compounds. An endpoint can be defined as a variable reflecting macrophyte performance and development during and after exposure to a toxic compound. Endpoints used in standard OECD *Lemna* tests are growth and biomass. Using submersed macrophytes, a series of other endpoints for macrophyte development and fitness can be considered.

Recently, the Species Sensitivity Distribution approach has been successfully applied to present the sensitivity of macrophyte species to different compounds and to compare these sensitivities with those of other taxonomic groups (Brock et al., 2004; Cedergreen et al., 2004a; Van Den Brink et al., 2006). Since the number of species included in our study was limited and this number did not meet the minimum number required for Species Sensitivity Distribution calculations, we used the sensitivity distribution concept to visualize the sensitivity of endpoints for a single macrophyte species to a compound and to compare sensitivity across endpoints and macrophytes. The method can be regarded as having been adapted from Hanson and Solomon (2002), as they present effect measure distributions for single plant species.

The aim of this paper was to analyse the toxicological sensitivity of macrophytes and macrophyte endpoints to toxicants with different modes of action (two fungicides, a herbicide and a biocide) in chronic, non-axenic laboratory tests. The analysis was intended to examine which macrophytes and which endpoints are toxicologically most sensitive and have an acceptably low variation between plants, and are therefore to be recommended for laboratory macrophyte toxicity tests.

2. Materials and methods

2.1. Laboratory macrophyte toxicity tests

Plant material was collected from stock populations in outdoor experimental ditches (length 40 m; width 1.5 m at the bottom; depth 0.8 m; clay sediment covered by a thin detritus layer) (Drent and Kersting, 1993) and mesocosms (diameter 1.8 m; depth 0.8 m; black HPE; clay sediment covered by a top layer of fine organic, eutrophic, uncontaminated mud). The material was collected about one week prior to the start of the experiments, to allow the macrophytes to acclimatize in test water (storage basin water from 'De Sinderhoeve' experimental station) (Drent and Kersting, 1993) in aquaria in a climate chamber. Tests were performed with *Myriophyllum spicatum* L., *Elodea canadensis* Michx., *Elodea nuttallii* (Planch.) H. St. John, *Ranunculus circinatus* Sibth. and *Potamogeton crispus* L. *M. spicatum* and *R. circinatus* are dicotyledonous species, while the other three are monocotyledons.

The macrophytes were incubated in a growth solution as top shoots with a length of approximately 8–10 cm, with similar wet weight per species at the start and without roots. No sediment compartment was added to the test vessels and macrophytes could freely float in the growth solution. Macrophytes were exposed to a range of toxicant concentrations for 21 days. Tests were performed in duplicate in glass vessels with a volume of 1.5 L. Experiments were carried out in a climate controlled room with an air temperature of 20 °C. Test vessels were illuminated by three daylight lamps (Philips HPI-T, 400 W lamps) producing 220 ± 20 μmol m^{-2} s^{-1} in a light regime of 14 h light and 10 h darkness. To ensure that all test vessels received the same amount of light, they were randomly moved around at regular time intervals.

Test vessels were filled with 1.2 L of test medium (to a water depth of 12 cm). Test water was a mixture of ground water and rain water and was obtained from the storage basin at 'De Sinderhoeve' experimental station. Test water was filtered with a 45 μm net to remove large particles. Nutrients were added from a stock solution to obtain the following nutrient concentrations in the test medium: N 0.0357 mM/L, P 0.0023 mM/L and C 0.0067 mM/L (added as NH_4NO_3, K_2HPO_4 and $NaHCO_3$, respectively) and 0.10 mL/L Tropica Mastergrow (containing the required amounts of micronutrients/elements: K 0.79, Mg 0.39, S 1.01, B 0.004, Cu 0.006, Fe 0.07, Mn 0.04, Mo 0.002 and Zn 0.002 (%, w/w)). During the test, 1 mL of the stock nutrient solution was added to each vessel twice a week to realize a more even nutrient supply in time. Demineralized water was added once a week to compensate evaporation losses. To control the stability of abiotic conditions in the test vessels, temperature/pH and oxygen concentrations were measured with a WTW pH323 meter (equipped with a Sentix pH electrode) and YSI model 58 Oxygen meter. Measurements were performed at the start of the experiment ($t = 0$ days), and at $t = 1$, 2, 7, 14 and 20 days.

Thirteen macrophyte endpoints covering growth, shoot and root development were measured (Table 1). To monitor macrophyte performance in the laboratory tests, relative growth rate (RGR) was calculated on the basis of the biomass in the control vessels according to Eq. (1).

$$\mathrm{RGR} = (\ln(Xt) - \ln(X_0))/T \quad (1)$$

where Xt = endpoint value at the end of the experiment (21 days); X_0 = endpoint value at the start of the experiment (0 days); T = test duration (21 days).

Table 1
Endpoints studied

Endpoint number	Endpoint
1	Dry weight (g)
2	Growth (g)
3	Relative growth (%)
4	Dry weight of roots (g)
5	Total growth (incl. roots) (g)
6	Relative total growth (%)
7	Length of main shoot (cm)
8	Length of new shoots (cm)
9	Total length of shoot (cm)
10	Number of new shoots
11	Number of new roots
12	Total length of roots (cm)
13	Average root length (cm)