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Comparing growth development of *Myriophyllum* spp. in laboratory and field experiments for ecotoxicological testing

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Abstract
Background, goals and scope Risk assessment of herbicides and the evaluation of contaminated sediments based on algae and the macrophyte *Lemna* sp. alone may underestimate the potential hazard of certain compounds. Therefore, various test systems with *Myriophyllum* spp. have been developed recently to assess the phytotoxicity in surface waters and natural sediments. In the present study, experiments investigating the growth development of *Myriophyllum* spp. were performed in the laboratory under defined conditions and in mesocosms under environmentally realistic exposure conditions to evaluate the suitability of these species as potential standard test organisms in ecotoxicological testing. This study provides data on the endpoints biomass, plant length and root development.
Materials and methods Six independent experiments were performed to investigate the plant development of *Myriophyllum* spp. under control conditions. The main difference in the experiments was the complexity of the test systems ranging from simple laboratory experiments to complex outdoor mesocosm studies. At the start of each experiment, uniform cuttings of *Myriophyllum* spp. were placed in vessels with or without sediments to reduce variability between replicates. The endpoints considered in this investigation were biomass (fresh weight of the whole plant), length of the main shoot, length of the side shoots, total length of the plant (calculated from the length of the main and side shoots) and root formation. Root to shoot ratios were calculated as a further measure for plant development. Relative growth rates (RGR) based on plant length (RG_LR) and on biomass (RG_BR) were calculated.
Results Despite the various experimental conditions, comparable growth was obtained in all test systems and the variability of endpoints, such as total length and biomass of plants, was low. It was observed that the RGR of *M. spicatum* in the simple laboratory test system with sediment were comparable to growth data obtained for *M. verticillatum* and *M. spicatum* grown in indoor and outdoor mesocosms, thus indicating that *Myriophyllum* growth tends to increase by the addition of sediment. High variability was determined for the endpoints length of the side shoots, total root length and biomass of roots.
Discussion One challenge for a test design to investigate phytotoxicity on aquatic plants is to obtain good growth of the plants. From the results, it can be concluded that the experimental conditions in the various test systems were suitable to study the plant development of *Myriophyllum* spp. because obtained growth rates were comparable between laboratory and field investigations. Another challenge for developing a plant biotest system is the definition of sensitive endpoints. Low variability is preferred to detect minor effects of chemicals or polluted sediments on plant development. In our studies, the variability of the endpoints biomass and total length of plant was low and, therefore, they have much potential as endpoints for assessing toxicity.

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Conclusions The methodologies presented in this study have applications within the risk assessment for aquatic plants and have the advantage of assessing effects taking into account the relevant exposure pathways via water and/or sediment for compounds under investigation.
Recommendations and perspectives Setting safe quality criteria for surface water and sediments is one of the challenges authorities are facing today. *Myriophyllum* spp. is recommended as suitable test species to investigate phytotoxicity in surface water and sediments. These results, thus, might serve as a basis for the compilation of a new harmonised guideline for ecotoxicological testing with aquatic macrophytes.

Keywords Aquatic plants · *Myriophyllum* · Plant development · Sediment contact test · Mesocosms · Ecotoxicological testing · Herbicide risk assessment

1 Introduction

Aquatic macrophytes play an important structural and functional role in freshwater ecosystems by influencing the carbon and nutrient cycle, forming primary productivity and providing food and habitat for other organisms. Especially in oligotrophic ponds and lakes, streams and wetland communities, submerged, floating, and emersed macrophyte species are essential to harbour diverse animal communities (Wetzel 2001). Regardless of the causes, any significant reduction in macrophytes can be expected to have a strong impact on the whole ecosystem (Lewis 1995).

Macrophytes are potentially endangered by high loads of nutrients and agricultural or urban chemicals, which may reach the aquatic environment by waste water discharge, pesticide run-off or spray drift. Both high nutrient and pesticide input into the surface waters has often been reported to cause a shift from macrophyte- to algal-dominated systems (Welch 1992; Van den Brink et al. 1997). Contaminated sediments may also play a key role in destabilising macrophyte communities (Lovett-Doust et al. 1994; Caffrey et al. 2006). Over the last decades, contamination of river and lake sediments has increased considerably. Sediments have been identified not only as a major sink for persistent toxic substances released into the aquatic environment, but also as a potential source (Brils 2002; Förstner 2004).

Phytotoxicity data are used for the development of water or sediment quality criteria and for the registration of pesticides (Klein et al. 1993). Regulatory interests in surface water and wetland protection and sediment quality criteria development result in an increased use of whole rooted plants as test species. To date, in Germany, the assessment of phytotoxic risk potential in sediments is exclusively based on the results of the algal test according to the German standard (DIN 38412 L33 1991) using pore water and/or eluates as a test medium. In the risk assessment of herbicides, algal tests (OECD 201 2002) and a standard macrophyte test with the duckweed *Lemna* sp. (OECD 221 2002) are required to assess the effects on autotrophic organisms.

However, macrophytes rarely play a role in regulatory or quality criteria decisions. This is in strong contrast to the ecological importance of macrophytes (Lewis 1995) and the European Water Framework Directive (http://ec.europa.eu/environment/water/water-framework/index_en.html), which considers macrophytes as indicators of water pollution at the same level as macrozoobenthos, fish and phytoplankton (for an overview, see Reiley et al. 2003). It is questionable if *Lemna* spp. as a monocot- and not sediment-exposed species can be seen as a representative for dicot- or sediment-rooting macrophytes. For some herbicides, such as auxins, laboratory data with algae and *Lemna* spp. were not sufficient to ensure protection of macrophytes (Brock et al. 2000; Davies et al. 2003; Cedergreen and Streibig 2005; Vervliet-Scheebaum et al. 2006). In these cases, further testing with additional species may be warranted to refine the risk assessment, but appropriate standard tests are lacking that address ecologically relevant endpoints for macrophytes. At present, there is only a standard procedure of the American Society for Testing and Materials (ASTM 1998) for aquatic macrophytes recommending the use of axenic cultures of the dicotyledonous submerged species *Myriophyllum sibiricum* Komarov. Because the use of axenic cultures is very time-consuming and the rooting substrate (Turface) recommended by the ASTM guideline has little sediment-like features, other test procedures are in development.

Myriophyllum spp. is widely considered as a suitable bioassay plant for the detection of herbicidal activity (Forney and Davis 1981; Paterson and Wright 1987; Selim et al. 1989; Green and Westerdahl 1990; Netherland and Getsinger 1992; Hanson et al. 2002, 2003; Turgut 2005) and has been proposed as a potential candidate for pesticide toxicity. A new bioassay with *M. spicatum* for evaluating phytotoxic effects in water or water–sediment systems was reported recently (Knauer et al. 2006). For the determination of phytotoxic effects in pure bulk sediments, a new sediment contact test with *M. aquaticum* was developed by Feiler et al. (2004). Various endpoints have been discussed for assessing toxic effects to freshwater plants, such as chlorophyll fluorescence, biomass and length of shoots and roots (Getsinger et al. 1982; Hanson et al. 2001; Feiler et al. 2004; Knauer et al. 2006; Arts et al. 2007; Küster et al. 2007).

We aimed to evaluate the suitability of *Myriophyllum* spp. as an additional test organism for future standard

guidelines to refine the risk assessment of herbicides and contaminated sediments. In this context, this paper compares the growth potential of *Myriophyllum* spp., as a prerequisite for the development of new tests, in different test systems and provides data on the endpoints biomass, plant length and root development. Experiments were performed in the laboratory under defined conditions and in mesocosms under environmentally realistic exposure conditions.

2 Materials and methods

2.1 Test designs and the respective test organisms

Six independent experiments were performed to investigate the plant development of *Myriophyllum* spp. under control conditions. The different experimental designs are summarised in Table 1. The main difference in the experiments was the complexity of the test systems. Starting with a "simple" system containing medium only (experiment 1), this proceeded to a test system containing the same medium and, in addition, artificial sediment (experiment 2), and a system containing sediment only (artificial or natural unpolluted sediment, experiments 3 and 4). Plant development was further investigated in complex indoor and outdoor mesocosm systems (experiments 5 and 6).

The dicotyledonous plant *Myriophyllum* spp. (Haloragaceae) was chosen as a test organism. Different *Myriophyllum* species were used in the experiments depending on the availability in the different laboratories and the access to suppliers. Features, which make these test organisms suitable for aquatic toxicity testing, are ease of culturing and their widespread and fast growing (Grace and Wetzel 1978; Barrat-Segretain 2004). *Myriophyllum* spp. can be rooted from vegetative cuttings. Those cuttings were taken for the experiments because seeds may often be less sensitive to chemicals that are present in their ambient environment (see Table 1; Pfleeger et al. 1991; Walsh and Weber 1991).

2.1.1 Myriophyllum spicatum

The plant development of the submerged *M. spicatum* L. was investigated in laboratory studies with M4 medium (experiment 1; Elendt 1990), with M4 medium supplemented by artificial sediment (experiment 2; OECD 218 2001) and in natural surface water and artificial substrate in an outdoor mesocosm study (experiment 6; Table 1).

Non-axenic cultures of *M. spicatum* were supplied as 10 to 15 cm long cuttings by the company Van der Valk

Table 1 Experimental designs for the laboratory (experiments 1–4) and the mesocosm studies (experiments 5 and 6)

Experiment	1	2	3	4	5	6
Test species	*M. spicatum*	*M. spicatum*	*M. aquaticum*	*M. aquaticum*	*M. verticillatum*	*M. spicatum*
Test system	1-l beaker	1-l beaker	1-l beaker	1-l beaker	15 m^3 indoor mesocosms	10 m^3 outdoor mesocosms
Test medium	M4	M4	–	–	Mesotrophic pond water	Mesotrophic pond water
Sediment	–	Artificial (OECD 218)	Artificial (OECD 207)	Natural	Sand/plant–soil mixture (2:1)	Artificial substrate
Growth behaviour	Submersed	Submersed	Emersed	Emersed	Submersed	Submersed
Light intensity (μmol m^{-2} s^{-1})	30–50	30–50	80–85	80–85	165	Daylight
Light regime	16 h/8 h day/night	16 h/8 h day/night	24 h continuous	24 h continuous	Monthly adapted to outdoor conditions	Natural light/dark regime
Temperature (°C)	23±2 (day) 20±2 (night)	23±2 (day) 20±2 (night)	24±2	24±2	range 12–20	range 8–16
pH	7.0±0.5	7.0±0.5	5.5±0.5	7.0±0.2	8.8±0.4	7.8–8.8
Initial average biomass per plant (mg wet weight)	100±30	110±30	32	38	450	175±20
Initial average length of cuttings (cm)	5	4	Whorls	Whorls	12	5
Plants per pots	1	1	7	7	7	2
Number of pots	10	5	3	3	2	12
Number of ponds	–	–	–	–	2	18
Test duration (days)	20	21	10	10	43/51	20
Endpoints	Biomass, shoot and root length	Biomass, shoot and root length	Biomass, length of the longest root	Biomass, length of the longest root	Biomass, length of shoot and longest root	Shoot and root biomass, shoot length

(garden and plants, Aesch, Switzerland). Pre-cultures were maintained in 20 l aquaria containing 8 l of M4 medium and maintained in a climate chamber with a photoperiod of 16 h light with a light intensity of 30–50 μmol m^{-2} s^{-1}. Temperature was maintained between 23±2°C during the day and 20±2°C during the night. The medium was renewed every second week. Detailed descriptions for experiments 1 and 2 were reported by Knauer et al. (2006).

The outdoor mesocosm experiment (experiment 6) was performed at the mesocosm test site of Syngenta Crop Protection in Stein, Switzerland. For this study, non-flowering plants were supplied by Alfred Forster AG (Golaten, Switzerland) and cut into 7-cm shoots. Two cuttings were inserted 2 cm deep into one plant pot containing artificial substrate to assure robust fixation. Twelve pots were then attached to a plastic box, which was placed into each mesocosm (Table 1). Plant development of the total 24 cuttings was studied; and biomass of the total plant, the shoots and the roots were determined at the start, during and at the end of the experiment (Table 1). For the length measurements, the plants had to be taken out of the water and the length of the main and side shoots was assessed.

2.1.2 *Myriophyllum aquaticum*

The emersed *M. aquaticum* was used in the sediment contact tests with artificial sediment (OECD 207 1984, experiment 3) saturated in Steinberg medium (ISO 20079 2005) and with natural unpolluted river sediments (experiment 4) from the old arm Altrip of the river Rhine (cf. Table 1). *M. aquaticum* was obtained as sterile culture from Jungnickel (University of Jena, Germany). Non-axenic pre-cultures of *M. aquaticum* were grown vegetatively in artificial sediment (OECD 207 1984) and saturated with Steinberg medium under defined growth conditions in a growth chamber.

For the experiments, defined whorls were cut from 21-day-old plants. The two to three whorls growing just below the head whorl of a plant were chosen to obtain homogeneous plant material. These whorls were randomly chosen and directly put into the sediment in the test vessels. Detailed descriptions of the sediment contact test were reported by Feiler et al. (2004).

2.1.3 *Myriophyllum verticillatum*

The submerged *M. verticillatum* was used in the indoor mesocosm study (experiment 5). *M. verticillatum* was harvested from an uncontaminated lake (Lake Britzer Garten, Berlin, Germany) and cultured in microcosms filled with uncontaminated sand and sediment and with a mixture of ground water and deionised water from the water works of the experimental field station of the Federal Environment Agency in Marienfelde, Berlin, Germany (http://www.umweltbundesamt.de/fsa). Nutrients were added biweekly to the microcosms to maintain nutrient levels of 2 mg l^{-1} Si, 1.5 mg l^{-1} total N and 0.04 mg l^{-1} total P.

For the experiment, seven 15-cm cuttings without side shoots were taken and inserted 3 cm deep into each plant pot filled with a sand/soil mixture of 2:1 (plant–soil= 100 mg l^{-1} N, 100 mg l^{-1} P; Stender). Two plant pots were placed on the sediment of each pond mesocosm at the deepest zone (100 cm below surface of the water). All plants of *M. verticillatum* from one plant pot were harvested at each sampling date. A detailed description of the experimental set-up of the indoor mesocosms (experiment 5) is given in Mohr et al. (2005, 2007).

2.2 Endpoints and calculations of variability

The endpoints considered in this investigation were biomass (fresh weight of the whole plant), length of the main shoot, length of the side shoots, total length of the plant (calculated from the length of the main and side shoots) and root formation (total length in experiments 1 and 2, length of the longest root in experiments 3, 4 and 5, biomass of roots in experiment 6; Table 1). Root to shoot ratios were calculated as a further measure for plant development. Biomass was determined as the wet weight at the start and end of each experiment. In experiment 5, an additional sample was taken during the experiment on day 43. Plants were harvested and not placed back in the mesocosm.

For the different endpoints of each experiment, the relative growth rates (RGR) were calculated over the duration of the experiments. The RGR based on plant length (RG_LR) and on biomass (RG_BR) for each replicate plant was calculated as follows:

$$\mathrm{RGR} = [\ln(X_t) - \ln(X_0)]/t \quad (1)$$

where X_t is the measurement of the endpoint at the end of the experiment (time t), X_0 is the measurement of the endpoint at the start of experiment (time 0) and t is the duration of the experiment (days).

The amount of random variability of each endpoint at each date and of the calculated RGRs for each experiment was determined using the coefficient of variation (CV). The CV was calculated as follows:

$$\mathrm{CV} = 100s/\overline{X} \quad (2)$$

where $\overline{X} = \left(\sum_{i=1}^{n} X_i\right)/n, s = \sqrt{\sum_{i=1}^{n} \left(X_i - \overline{X}\right)^2 \Big/ (n-1)}$, n is the number of replicate plants and X_i is the measurement of the endpoint for replicate plant i.

3 Results

3.1 Plant development in the various test systems

The relative growth rates of the different *Myriophyllum* species based on biomass (RG_BR) and total plant length (RG_LR) in the various test systems are shown in Table 2. The RG_BR of the submerged species *M. spicatum* and *M. verticillatum* ranged between 0.033 and 0.068 day^{-1} in medium containing test systems (experiments 1, 2, 5 and 6) and between 0.103 and 0.118 day^{-1} for the emersed species *M. aquaticum* in the test system containing sediment only (experiments 3 and 4). The lowest RG_BR was observed for *M. spicatum* in the M4 medium without sediment (experiment 1). The RG_BR increased by 30–110% when sediment was added to the systems (experiments 2, 5 and 6) and by approximately 260% in test systems containing sediment only (experiments 3 and 4).

The RG_LR of *M. spicatum* and *M. verticillatum* in experiments 1, 2, 5 and 6 ranged between 0.028 and 0.052 day^{-1}. The lowest RG_LR was again determined for *M. spicatum* in the test system containing M4 medium only (experiment 1). The addition of sediment to the M4 medium enhanced the growth by up to 85%. This, together with the observation of the RG_BR, indicates that *Myriophyllum* growth tends to increase by the addition of sediment. Furthermore, it was observed that the RGR of *M. spicatum* in the simple laboratory test system with sediment (experiment 2) were comparable to the growth data obtained for *M. verticillatum* and *M. spicatum* grown in indoor and outdoor mesocosms (Table 2). In sediment only systems (experiments 3 and 4), the RG_LR of *M. aquaticum* was highest. Data on total increase in biomass and plant length were comparable to data on growth rates indicating the importance of sediment in plant development (Table 2).

The number of side shoots was determined in experiments 1, 2, 3, 4 and 5. After 20–21 days, *M. spicatum* developed approximately 1.5 side shoots per plant, *M. verticillatum* approximately 2.3 side shoots per plant after 51 days, and *M. aquaticum* on average 2 side shoots per plant (Table 2). Root formation was studied in the test systems resulting in root to shoot ratios of 0.041–0.048 for *M. spicatum* after 20–21 days and 0.11 for *M. verticillatum* after 51 days (Table 2). The highest ratio was observed for *M. aquaticum* in the sediment only system (Table 2).

3.2 Variability of the different endpoints

The CVs allow the comparison of the variability between the different endpoints and experiments (Table 3). Variability was determined for the endpoints measured at the end of the experiment (Fig. 1) and for the RGR of the different endpoints (Fig. 2).

The lowest variability was observed for the endpoints biomass, total plant length and length of main shoot measured at the end of all experiments (Fig. 1). In contrast, high CVs were determined for the endpoint length of the side shoots. The CVs of the endpoint root formation were comparable and low in experiments 3–5 ranging between 18 and 24 in which the length of the main root was determined, whereas the CVs in experiment 1 and 6 determined as total root length and biomass of roots strongly varied with CVs ranging between 52% and 75% at the end of experimental duration (Fig. 1, Table 3).

The CVs for each sampling date within one experiment varied in the same order of magnitude except for the length of the side shoots (Table 3). The CVs of the side shoots decreased with increasing experimental duration (experiments 1, 2 and 6; Table 3).

All experiments showed low CVs based on RGR between 8% and 35% for the endpoints biomass and total plant length (Fig. 2). The RGR-based CVs for biomass were on average 0.6-fold lower than that from biomass data at the end of the experiments, whereas the CVs of RGR data for total length were similar to those of the total length of the plant at the end of the experiment (Table 3).

Table 2 Comparison of various growth parameters from *Myriophyllum* spp.

Experiment	Aquatic plant	Duration of experiment (days)	RG_BR (day^{-1})	RG_LR (day^{-1})	Number of roots	Total length of roots (cm)	Number of side shoots	Root to shoot ratio	Reference
1	*M. spicatum*	20	0.033±0.009	0.028±0.010	2.0 (±1.6)	4.1 (±3.0)	1.4±0.7	0.46[a]	Knauer et al. (2006)
2	*M. spicatum*	21	0.056±0.007	0.050±0.014	11 (±1.7)	5.1 (±1.3)	1.6±0.9	0.48[a]	Knauer et al. (2006)
3	*M. aquaticum*	10	0.103±0.014	0.178±0.004	6.6 (±1.7)	12.8 (±3.5)	2,0 (±0.5)	4.3[a]	This study
4	*M. aquaticum*	10	0.118±0.010	0.199±0.007	5.1 (±1.2)	14.8 (±3.1)	2.1 (±0.3)	4.0[a]	This study
5	*M. verticillatum*	51	0.043±0.010	0.052±0.005	n.d.	14.3 (±2.3)	2.3±0.7	0.11[a]	This study
6	*M. spicatum*	20	0.068±0.014	0.039±0.006	n.d.	n.d.	n.d.	0.41[b]	This study

n.d.: not determined, RG_LR: relative growth rate based on total length, RG_BR: relative growth rate based on biomass
[a] Based on length measurements.
[b] Based on biomass.

Table 3 CV for each time point of the various endpoints and for the RGR of individual plants of *Myriophyllum* spp.

Experiment	Time (days)	Biomass	Total length of plant (cm)	Length of main shoot (cm)	Length of side shoots (cm)	Root formation
1	0	33	0	0	0	0[a]
	7	n.d.	11	8	124	86[a]
	9	n.d.	14	10	103	63[a]
	13	n.d.	18	12	90	65[a]
	17	n.d.	18	13	72	71[a]
	20	34	20	12	68	75[a]
	RGR	27	35	45	67	n.d.
2	0	30	0	0	0	n.d.
	3	n.d.	8	6	224	
	6	n.d.	21	6	157	
	8	n.d.	15	16	157	
	10	n.d.	22	17	66	
	13	n.d.	20	17	32	
	15	n.d.	20	17	37	
	17	n.d.	26	21	50	
	21	37	25	21	52	
	RGR	12	27	68	34	
3	0	9	0	n.d.	n.d.	0
	10	16	24			23[b]
	RGR	14	3			n. d.
4	0	12	0	n.d.	n.d.	0
	10	15	23			24[b]
	RGR	8	4			n. d.
5	0	n.d.	0	0	0	0
	43	34	17	21	61	18[b]
	51	28	29	8	84	18[b]
	RGR	18	6	8	n.d.	n.d.
6	0	10	0	0	0	0
	6	n.d.	9	6	312	n.d.
	13	n.d.	17	9	159	n.d.
	20	29	19	9	103	52[c]
	RGR	21	16	11	41	n.d.

n.d.: not determined
[a] Total length of roots (cm).
[b] Length of longest root (cm).
[c] Wet weight of total roots (mg).

However, RGR data obtained with the endpoint length of the main shoot showed variability in experiments 1 and 2, being on average 3.5-fold higher than data obtained at the end of the experiment, but similar CV values in experiments 5 and 6. CVs calculated from the RGR data of the side shoots were lower than those calculated for each parameter during the experiment (Table 3).

4 Discussion

One challenge for a test design to investigate phytotoxicity on aquatic plants is to obtain good growth of the plants. Optimal light and nutrient conditions should be chosen to produce optimal growth of the plants. From the data presented in Table 2, it can be concluded that the experimental conditions in the various test systems were suitable to study the plant development of *Myriophyllum* spp. The obtained growth rates were comparable between laboratory and field investigations. The high growth rates in the sediment only experiments with *M. aquaticum* might be explained by higher light intensity and longer light cycle (cf. Tables 1 and 2). Growth results presented in this study were similar to published data (Forney and Davis 1981; Getsinger et al. 1982; Turgut and Fomin 2001; Hanson et al. 2001, 2003; Stesevic et al. 2007). In terms of the parameter growth rate, *Myriophyllum* spp. seems to be a suitable test organism to assess the phytotoxicity of herbicides or contaminated sediments.

Another challenge for developing a plant biotest system is the definition of sensitive endpoints. When performing tests with living organisms, one has to deal with natural

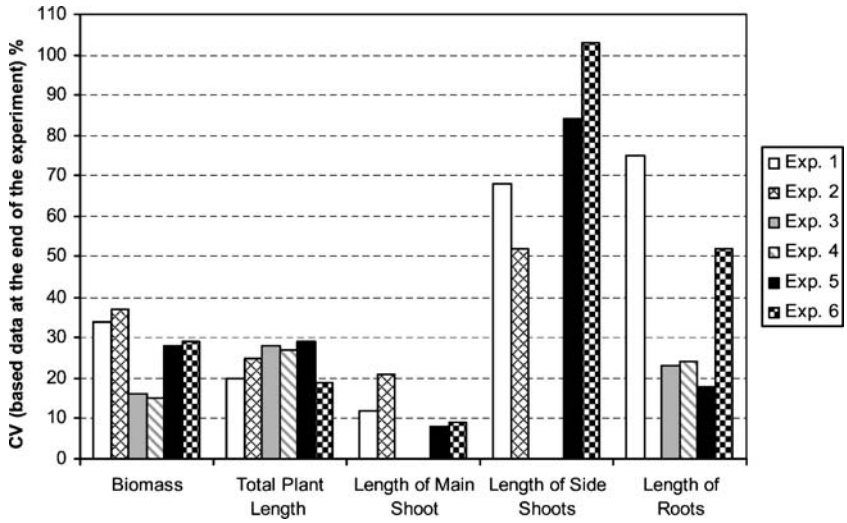

Fig. 1 Comparison of the CVs of all endpoints measured at the end of the experiments

variability due to genetic differences and to differences between different laboratories. The CV (variability) gives information on the stability of the chosen endpoints. Low variability is preferred to detect minor effects of chemicals or polluted sediments on plant development. In our studies, the variability of the endpoints biomass and total length of plant was low throughout each experiment. Neither the used species nor the used test design influenced this result drastically. The use of taking uniform cuttings from stock cultures is, therefore, strongly recommended. Comparable variability of the endpoints biomass and total length of plant were also described for *M. sibiricum* (Getsinger et al. 1982; Roshon et al. 1999) and *M. aquaticum* (Turgut and Fomin 2001). The development of many side shoots may hamper the results as this endpoint showed highest variability. However, this had only a minor effect on the variability of the total plant length calculated by summing up the length of the side shoots and the length of the main shoot. A high variability of the endpoint length of the side shoots was also described for *M. aquaticum* by Turgut and Fomin (2001).

The variability of the endpoint root formation was low in experiments 3, 4 and 5 where only the longest root was measured, whereas it was high in experiments 1 and 6 where either total root length or root biomass was calculated. Although the variation is low, it is questionable whether it is useful to choose only the longest root as an indicator for root development because dicot plants usually develop homorhizy. On the other hand, due to its time-consuming effort, it is not feasible to measure all roots. Thus, if root formation should be used as an endpoint, we recommend measuring the biomass of the roots instead of root length. High variability in root length between replicated plants was also observed by Sanchez et al. (2007) and Arts et al. (2007). Arts et al. (2007) presented the root growth as a very sensitive endpoint in some toxicity studies, but investigated plant growth in test systems including medium only. The test system without

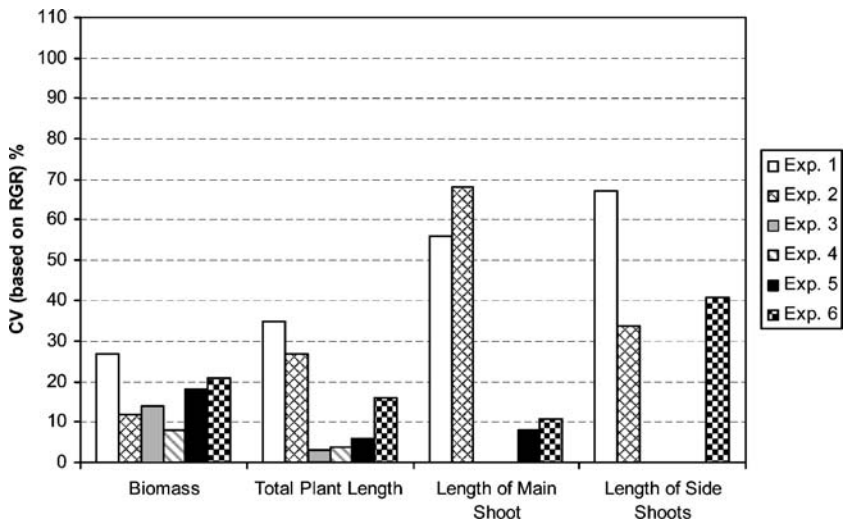

Fig. 2 Comparison of the CVs of the RGR calculated from various endpoints

substrate might be suitable for herbicides with very high water solubility. However, for other compounds, which highly adsorb to the sediment, we recommend adding a sediment phase to the test system enhancing the realism of the test system. In this case, the observation of the root development, however, becomes very time-consuming and variable because of practical problems as presented in this study (Table 3). It is, therefore, worth striving for a test system in which both options, with or without sediment, can be performed to investigate chemicals with different properties.

The root to shoot ratio of *M. spicatum* was high in comparison to observations for *M. spicatum* in natural communities where a ratio of 0.01–0.15 was observed at the seasonal biomass maximum (Grace and Wetzel 1978). Data obtained from *M. verticillatum* in mesocosms, however, was comparable to this field observation.

The comparison of the different test designs in this study demonstrated that differences in the growth of *Myriophyllum* spp. were rather linked to the presence of sediment and higher light conditions than to the different degrees of complexity of the test systems. The advantage of laboratory toxicity tests is that they can be performed at highly controllable and reproducible conditions and low costs, whereas mesocosm studies reflect more environmentally realistic conditions, but are time-consuming and cost-intensive. In this study, we demonstrated that both the simple and complex experimental set-ups resulted in reliable results. The choice of an appropriate test system, however, should always be based on the risk assessment question. The sediment contact test evaluating the growth of the emersed *M. aquaticum* based on biomass should be chosen if the impact of contaminated sediments is of concern. As a tier 1 test, the simple laboratory test investigating shoot growth of *M. spicatum* is recommended to be suitable for the determination of the toxicity of herbicides. For a further refinement of the risk posed by herbicides, the evaluation of the growth of *Myriophyllum* sp. in mesocosm studies is feasible.

For regulatory purposes, it is essential that bioassays are able to confidently detect changes caused by anthropogenic pollution. Given the restrictions applied by a feasible number of replicates, the increase in replicate number to more than five in laboratory studies and three in mesocosm studies using five plant pots is probably not realistic. Under these experimental conditions, endpoints such as total plant length, length of main shoot and fresh weight appear to combine low statistical variability and ecological relevance and are, therefore, suitable endpoints for investigating plant development. This has been demonstrated in several studies such as the sediment contact test with *M. aquaticum*, which was effectively applied for the assessment of toxic effects in natural sediment samples (Feiler et al. 2004; Stesevic et al. 2007). Growth rate data based on biomass was used in these experiments as the appropriate endpoint. Furthermore, in a mesocosm study with the biocide Irgarol, plant length was the most reliable endpoint and *M. verticillatum*, in comparison to other macrophytes, was the most sensitive plant (UBA 2007).

5 Conclusions

Setting safe quality criteria for surface water and sediments is one of the challenges authorities are facing today. For the most part, current criteria in aquatic risk assessment do not sufficiently address phytotoxicity. With the phylogenetic and morphological diversity among aquatic plants, it is unlikely that the current standardised test methods with algae and *Lemna* are protective for aquatic plants. The different methodologies presented in this study have applications within the risk assessment for aquatic plants and have the advantage of assessing effects taking into account the relevant exposure pathway via water and/or sediment for compounds under investigation.

6 Recommendations and perspectives

Due to the results on growth, we recommend *Myriophyllum* spp. as a suitable test species for assessing the phytotoxicity of herbicides or contaminated sediments. Biomass and total plant length appeared to be the parameters with lowest CVs and are, therefore, recommended as suitable test endpoints. Low variation is necessary to detect minor effects of chemicals in water or sediments on plant development. Due to its emersed growth, *M. aquaticum* is especially suited for investigating naturally polluted sediments. These results, thus, might serve as the basis for the compilation of a new harmonised guideline for ecotoxicological testing with aquatic macrophytes as is requested by the scientific community (discussed at the AMRAP workshop, Wageningen, The Netherlands, 14–16 January 2008). As further steps, the sensitivity to various chemicals and contaminated sediments of the proposed *Myriophyllum* species has to be investigated.

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