Sediment contact test with *Myriophyllum aquaticum* (ISO/DIS 16191): results of an international ring test

Ute Feiler*[1], Arts G[2], Bazin C[3], Belgers D[2], Biermann U[4], Brauer F[5], Casado C[6], Dören L[7], Eklund B[8], Gilberg D[9], Grote M[10], Hafner C[11], Knezevic V[12], Kopf W[13], Lemnitzer B[4], LePage G[14], Liedtke A[15], Matthias U[16], Okos E[1], Pandard P[17], Peter A[18], Ratte HT[19], Scheerbaum D[18], Schmitt-Jansen M[20], Stewart K[14], Teodorovic I[12], Weber K[21], Wenzel A[22], Ratte M[19]

[1]Federal Institute of Hydrology (D), [2]Alterra (NL), [3]Insavalor Polden (F), [4]Appelt Laboratorien (D), [5]UBA (D), [6]Centre Ecotox (CH), [7]Mesocosm GmbH (D), [8]Uni Stockholm (S), [9]ECT (D), [10]EDF (F), [11]Hydrotox (D), [12]Uni Novi Sad (SRB), [13]LfU Bayern (D), [14]AstraZeneca (UK), [15]Harlan Laboratories (CH), [16]LUBW (D), [17]INERIS (F), [18]Noack-Laboratorien (D), [19]ToxRat Solutions (D), [20]UFZ (D), [21]Eurofins (D), [22]IME-Fraunhofer (D)

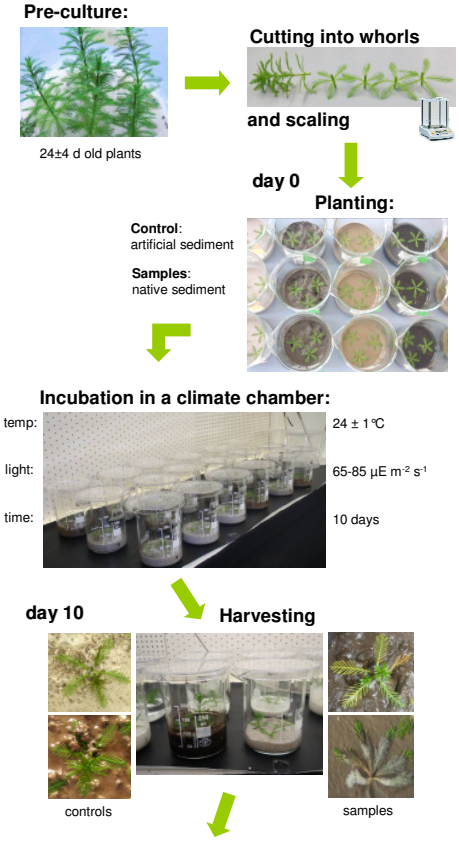
The contact test with the aquatic plant *M. aquaticum*[a] represents the exposure pathway via pore water and particle contact, and serves for the determination of growth-inhibitory effects of phytotoxic substances in environmental samples on *M. aquaticum* as a representative of the producer level. Although they are an important part of an aquatic ecosystem, dicotyledonous macrophytes are not yet part of the risk assessment of sediments and dredged material.

The contact test is now under standardization within ISO (→ ISO/DIS 16191) and a international ring test was performed. The ring test aimed at: 1) investigating the practicability and reproducibility of the sediment contact test, 2) validating the validity criterion (minimum growth rate), and 3) determining the response range of the reference substance.

Why sediment contact tests ?

- Sediments play a key role in the assessment of the ecological status of waters (habitats of an abundant biocoenosis / places for multitude biochemical transformations).
- Sediment studies are suitable for indicating the presence of anthropogenic pollution in waters.
- Whole-sediment exposure protocols → most realistic scenario !

ISO/DIS 16191 procedure:



$$\mu = \frac{\ln FW_{t2} - \ln FW_{t1}}{t_2 - t_1}$$

Figure 1: Sediment contact test with *M. aquaticum* (ISO/DIS 16191)

Investigated sediments

- *sample 1*: artificial sediment (control sample)
- *sample 2*: artificial sediment prepared by adding acetone (solvent control)
- *sample 3*: artificial sediment prepared by adding 90mg/kg 3,5-dichlorophenol (reference substance)
- *sample 4*: native sediment prepared by adding 800mg/kg nickel chloride (example for medium contaminated sediment)

Statistics

Repeatability:

$$CV_r\ \% = (s_r / \bar{X}_r)*100$$

CV_r, intra laboratory coefficient of variation
X_r, laboratory mean (growth rates)
s_r, standard deviation of laboratory mean

Reproducibility:

$$CV_R\ \% = (s_R / \bar{X}_R)*100$$

CV_R, inter laboratory coefficient of variation
X_R, mean of all replicates (growth rates)
S_R, standard deviation of all replicates

Median absolute deviation (MAD):

$$MAD = median_i\ (|x_i - median_j\ (x_j)|)$$
$$MAD\% = MAD/median_j\ (x_j)\ *100$$

$X_{i,j}$, single inhibition value
$median_{i,j}$, median of inhibition values

Summary of results

Table 1: Interlaboratory test results – toxicity parameters

sample	parameter	n[a]	mean	S_R	CV_R %	CV_r %
1	growth rate	14	0,135	0,028	21	7,0
2	growth rate	14	0,133	0,030	22	9,7
3	growth rate	14	0,090	0,023	25	10,6
4	growth rate	14	0,102	0,025	25	13,7
sample	**parameter**	**n[a]**	**median**		**MAD[b] %**	
3	inhibition	14	33,7		26,7	
4	inhibition	14	24,3		39,9	

[a]: valid and ISO-conform data
[b]: MAD = median absolute deviation from median, calculated considering non-normally distributed data showing no homogeneity of variance like inhibition values, MAD% = percentage MAD, measure of reproducibility (≙ CV_R%)

Results of the international ring test

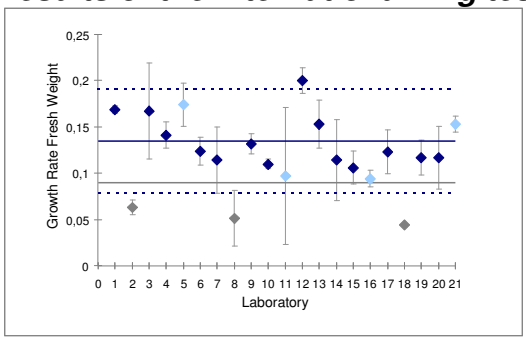


Figure 2: Myriophyllum growth rate in control sediment
◆ valid ◆ valid but deviations from ISO-protocol; ◆ non valid (growth rate < 0,09 d^{-1}); single values with confidence interval; ─ mean; -- 95% prediction interval, based only on valid data; ─ validity criterion (µ ≥ 0,09 d^{-1})

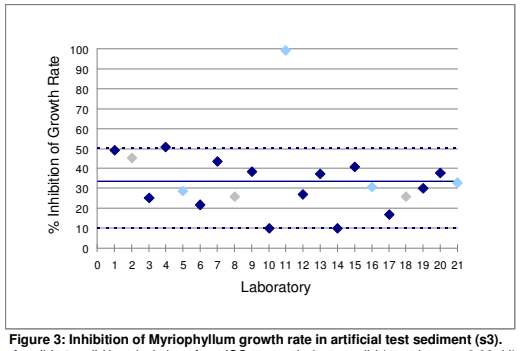


Figure 3: Inhibition of Myriophyllum growth rate in artificial test sediment (s3).
◆ valid; ◆ valid but deviations from ISO-protocol; ◆ non valid (growth rate < 0,09 d^{-1}); ─ median; -- 95% prediction interval, calculates as 2,5% & 97,5% quantiles, based only on valid data;

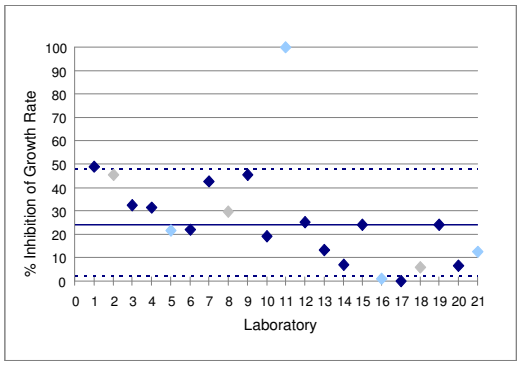


Figure 4: Inhibition of Myriophyllum growth rate in native test sediment (s4).
◆ valid; ◆ valid but deviations from ISO-protocol; ◆ non valid (growth rate < 0,09 d^{-1}); ─ median; -- 95% prediction interval, calculates as 2,5% & 97,5% quantiles, based only on valid data;

Conclusion

- Low CV_r (intra-laboratory variability (repeatability)) → stable test system
- Acceptable CV_R (inter-laboratory variability (reproducibility)) → useful as standardized method
- The proposed validity criterion of µ ≥ 0,09 is ok
- An additional validity criterion of CV_r<15% in the controls is recommended
- Reference substance: 90 mg 3,5-DCP/kg dw results in 35±15% inhibition
- Aceton is suited as solvent, it did not significantly impact the growth rate

(a) Feiler et al., 2004, JSS 4, 261-266

*contact: feiler@bafg.de