

# Observation Parameters of the Duckweed Growth Inhibition Test

## FronD number - Total FronD Area - Dry weight

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The classical growth observation parameter of the duckweed growth inhibition test is the total number of visible fronds. The frond number can be assessed without any technical tools, but considerable amount of time and limited ecotoxicological relevance.

Taking into account an increasing number of tests and small percentages of inhibition - i.e. a high number of fronds per vessel - manual counting is revealed to be quite a factor of time and expense. The LemnaTec Scanalyzer automatically counts the fronds and quantifies colour-parameters like chlorosis and necrosis faster, much more objective and reproducible without additional work. Beyond, this technical equipment for image analysis gives a comfortable assessment of total frond area. The assessment of total frond area is another growth parameter proposed for example in the OECD-guideline on duckweed. But assessing the total frond area has a number of further test-specific advantages.

While counting evaluates barely visible fronds the same way as fully-grown ones, looking at the total frond area weights the fronds proportionally to their individual area. This is much closer to a measurement of biomass than frondcounting. If the inhibition is calculated via growth rate, total frond area as the parameter of observation shows another structural advantage. While the total frond area grows continuously and independently of the number of observed fronds, within the first days of a test the frond number growth with an unrealistic high growth rate (Fig. 1a) and high standard deviations. The reason for this lies in an artefact of the test-design.

The inoculum generally consists of a 4 - 6 colonies with 2 or 3 large or medium sized fronds. As every frond has two pouches for young fronds, a large number of small fronds become visible within some hours after the start of the test. This effect is intensified cell-elongation caused by stress. So in the first days average growth rate of frond number is generally higher than growth rate of total frond

area. Growth rates of total frond area remain stable over the whole test if no lag-phases or toxic effects occur (Fig. 1 and 2).

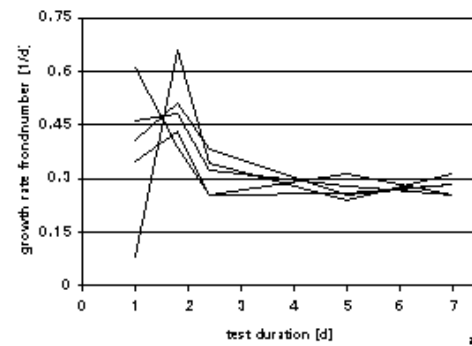


Fig. 1: Segmented growth rates for frond number (a) and total frond area (b) for a 7 days test with 5 control replicates (Lemna minor, Steinberg medium).

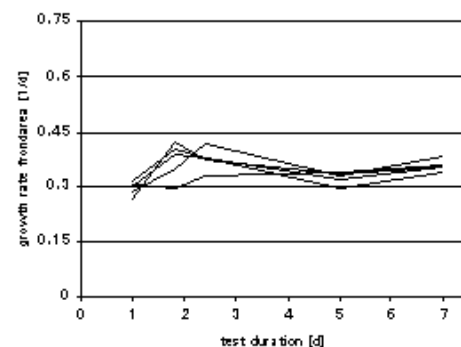


Fig. 2: Segmented growth rates for frond number (a) and total frond area (b) for a 7 days test with 5 control replicates (Lemna minor, Steinberg medium).

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This artefact of measurement makes it more difficult to show significantly that the control group grows exponential if only frond numbers are measured causing poor  $r^2$  values for linear regression. Furthermore the overestimation of initial growth rate using only frond numbers disguises lag-phases which could affect test validity.

Many toxicants - e.g. chromate - lead to a reduction of average single frond area. As a consequence frond area generally leads to higher inhibition values than inhibition of frond number.

Fig. 3 shows the concentration-inhibition dependence for potassium dichromate with frond number, dry weight and total frond area as parameters of observation. The inhibition of frond area is up to 20 % higher than the equivalent value for the frond number.

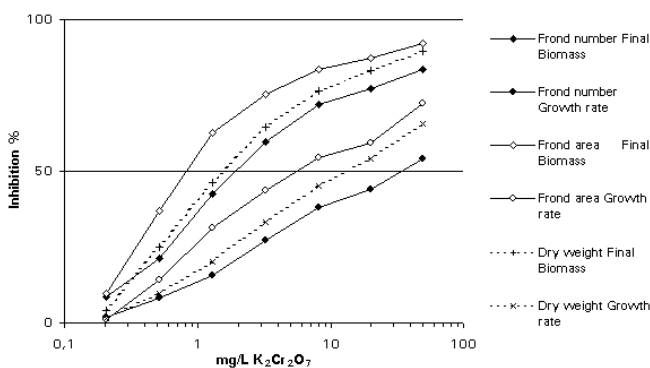


Fig. 3: Inhibition of duckweed frond number, dry weight and total frond area as a function of concentration of potassium dichromate. Inhibition values are calculated by final biomass and growth rates.

Using the LemnaTec Scanalyzer, the distribution of single frond areas are assessed in addition to total frond area and frond number. This gives valuable additional information. With rising concentration the maximum of frond area distribution shifts to lower values (Fig. 4).

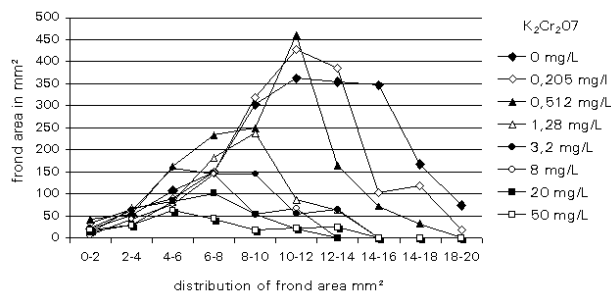


Fig. 4: Frond area distribution for different concentrations of potassium dichromate.

As a further consequence of mean frond area reduction, EC-values for the frond number are higher than those for frond area by a factor 1,5 to 6 (Table 1).

		EC20 [mg/L K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> ]		EC50 [mg/L K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> ]	
		final biomass	growth rate	f. biomass	growth rate
Total area	frond	0,3	0,8	0,9	6,1
Dry weight		0,4	1,2	1,7	14,2
Frond number		0,5	2,0	2,2	37,0

Fig. 5: Tab. 1 EC-values for the same test depending on the observation parameter and method of calculation.

Dry-weight as another classic biomass parameter leads to EC-values higher than those for frond area. Another disadvantage of dry-weight is, that wet-handling and drying needs further human contact to the duckweed and toxicants.

## Summary

With the aid of the LemnaTec Scanalyzer single and total frond areas are assessed fast, objectively and without destruction. In combination with automatically counted fronds, growth is measured comprehensively and in compliance with all international standards.

Total frond area leads to similar or smaller EC-values than frond number and dry-weight. The OECD-guideline and DIN-standard will support this development of automated detection of parameters by image processing.

Using the LemnaTec Scanalyzer to assess frond area in combination with other automatically assessed parameters like frond number and colour (chlorosis and necrosis) image analysis leads to a comprehensive, efficient and reproducible test documentation.