



Observation Parameters in Duckweed Growth Inhibition Tests

LemnaTec GmbH
Matthias Eberius
Schumanstr. 18
52146 Würselen, Germany
Tel. + 49 (0) 2405 / 4126-0
Fax + 49 (0) 2405 / 4126-26
matthias.eberius@lemnatec.de
www.lemnatec.de

The Colour of Duckweed

The colour of a plant, in this case duckweed, is equally important for the assessment of plant vitality as the number and area of fronds and their habitus. Often chlorotic and necrotic areas, but also fronds coloured in a lighter green, occur in bio testing besides a reduction of frond area and number, showing that substances may be having a direct or indirect negative influence on the photosystem. But even intensification of the green colour – which occurs for example with low concentrations of triazines – may indicate that a plant tries to compensate a toxic impact on its cells. Without employment of an image analysis system, the gradual colour classification of vitality in bio tests can be done by the following three methods:

1) Measurement of extracted chlorophyll

At the end of the test the duckweed needs to be crushed in hot ethanol and extracted overnight. The next day, the chlorophyll is quantified photometrically. This analysis requires additional work and good laboratory skills, but if all conditions remain constant, the results lead to a sensible and reproducible toxic endpoint. However, as the measurement is destructive and no aliquots can be made, there are no curves of growth to be obtained and no chances to reanalyse results or do multiple measurements. And a deepened colour may be distinguished from a larger amount of biomass only by simultaneously observing the total frond area.

2) Manual report

According to test guidelines, all changes in the test organisms should be noted at each measurement point. This kind of qualitative description is highly subjective and significantly increases the time needed. But nevertheless, these data are not integrated in toxicological endpoints making any changes if observed or not. Furthermore, later reanalysis or reconstruction of the original test event remains difficult because of the subjective classification of fronds.

3) Visual frond classification

To avoid assessment of healthy, damaged and dead fronds at the same level, ASTM and OPPTS Guidelines have specified that only healthy fronds with less than 50 % chlorosis are to be counted as living. On one hand this enhances the counting, but on the other hand it results in a further increase in time and a significant amount of subjective influences.

The LemnaTec Scanalyzer – an Ideal Tool for Colour Image Analysis

Image analysis systems are far superior to human eyes where the reproducible detection and quantitative classification of colours is concerned. But it is very important to transfer the colour information of every image pixel into ecotoxicologically relevant information. Image processing offers 4 different approaches to classify and quantify the colour of duckweed.

1) Distribution of total frond area colours

To assess the front vitality, the colours of all pixels are divided into colour classes, corresponding for example to healthy (green), lightly damaged (pale

green), chlorotic (yellow) and necrotic (grey or brown) frond areas. Without additional effort this method leads to an objective and standardised assessment of chlorotic and necrotic structures in relation to the influence of the testing material (cp. fig.1).

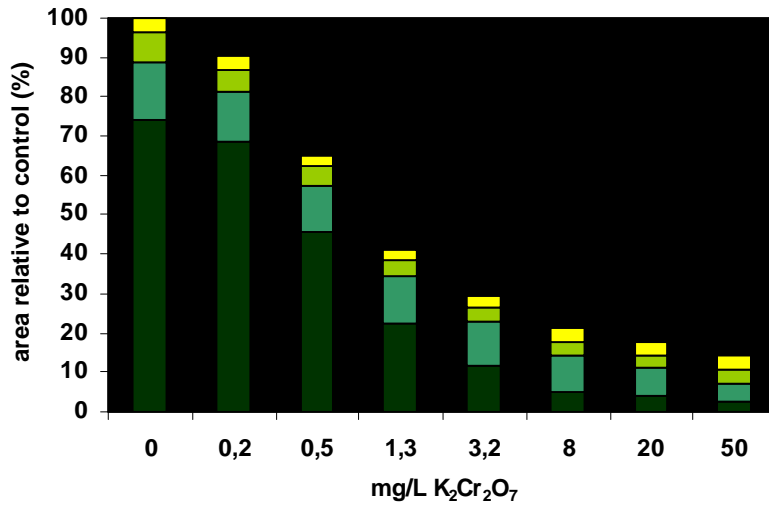


Fig. 1: Change in the areas of different colour classes with rising concentration of potassiumdichromate.

2) Colour classes and single fronds

The LemnaTec Scanalyzer is able to separate single fronds, for each of which colour classes can be determined simultaneously. The resulting amount of information can be handled by correlating colour classification and distribution of frond area. The result is an objective and evident information if small or fully grown fronds are particularly damaged. Final reports can then be completed on a well-documented basis. Fig. 2 shows the different steps from original image to frond area distribution combined with colour class information. Particularly younger fronds and very large fronds are damaged by chlorosis and necrosis.

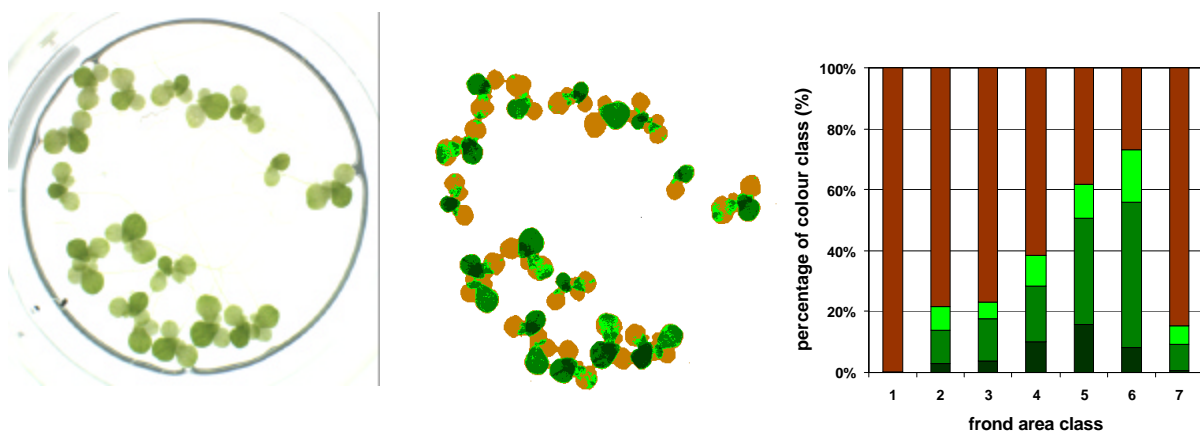


Fig. 2: Original image, colour class image and frond area distribution combined with colour classes. Brown colour represents chlorosis and necrosis.

3) Frond classification

Following the ASTM Guideline, image analysis fully supports the classification of fronds in living and dead. For this purpose merely the percentage of colour classes has to be adapted according to the guideline. Without further effort the fronds can be classified in living and dead, using data for the calculation of growth inhibition or rates of death.

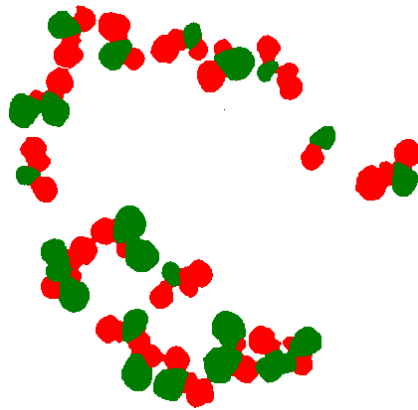


Fig.3: Classification of fronds in living and dead (more than 50 percent chlorosis or necrosis)

4) Specific green values

If colour analysis is made in accurate steps and the results are correlated with chlorophyll, an "image analysis green value" can be assessed. This provides a value similar to chlorophyll contents without the extra work and – more importantly – is a non-destructive method, at the same time making it possible to use this sensitive endpoint in kinetic studies. Like chlorophyll contents, green values integrate vitality in a single value for each treatment .

Summary

Colour is an important parameter to assess vitality of duckweed in ecotoxicological test systems. The LemnaTec Scanalyzer provides the unique opportunity to use relevant colour information in compliance with international standards. Informative colour quantification combined with analysis of frond area and number leads to comprehensive, objective and convenient test documentation of toxicity assessments.

For further information please do not hesitate to contact

LemnaTec GmbH
Matthias Eberius
Schumanstr. 18
52146 Würselen, Germany
Tel. +49 (0) 2405 / 4126-0
Fax +49 (0) 2405 / 4126-26
matthias.eberius@lemnatec.de
info@lemnatec.com
www.lemnatec.de