



## LemnaTec Collembol

Evaluation of Collembolate tests with the  
LemnaTec Scanalyzer

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## **Biological background**

With the increasing national and international legislation of environment and soil protection, ecotoxicological biotests with soil organisms become more important. The ISO standard 11267 "Soil quality – Inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants" (ISO 1999) as an international standard for the test of chemicals, forms the basis of the collembolatest. Considering the fact of some special features (Fraunhofer 2001b) the standard can be used for testing contaminated soils. For this purpose a ringtest has been performed successfully (Fraunhofer 2001a). Within the framework of a following inter laboratory test of 8 different contaminated soils, 5 of the soils were assessed as toxic. Because of these results, the collembolatest was suggested to be one of 5 tests within the test strategy for the assessment of habitat function (Fraunhofer 2001b).

## **Implementation of the Collembolatest**

The standard intends, next to an acute test for the pre-test, a chronic 28 day reproduction test. For the chronic test, at the test start 10 animals, between 10 and 12 days old are put into the soil probe and 28 days later the test must be aborted. For the evaluation the organisms and soil is put into water. Ink is added to this water for a better contrast. Because dead Collembola and shed skins are losing their hydrophobic characteristic and are eaten by other Collembola as destructive organisms, almost only living organisms float on the water surface. At the test end up to 1000 Collembola per vessel must be counted. This means up to 20 000 Collembola for an average test. These are often recorded in double counts, because corresponding to the norm it is not allowed to exceed a count error of 10 % (ISO 1999).

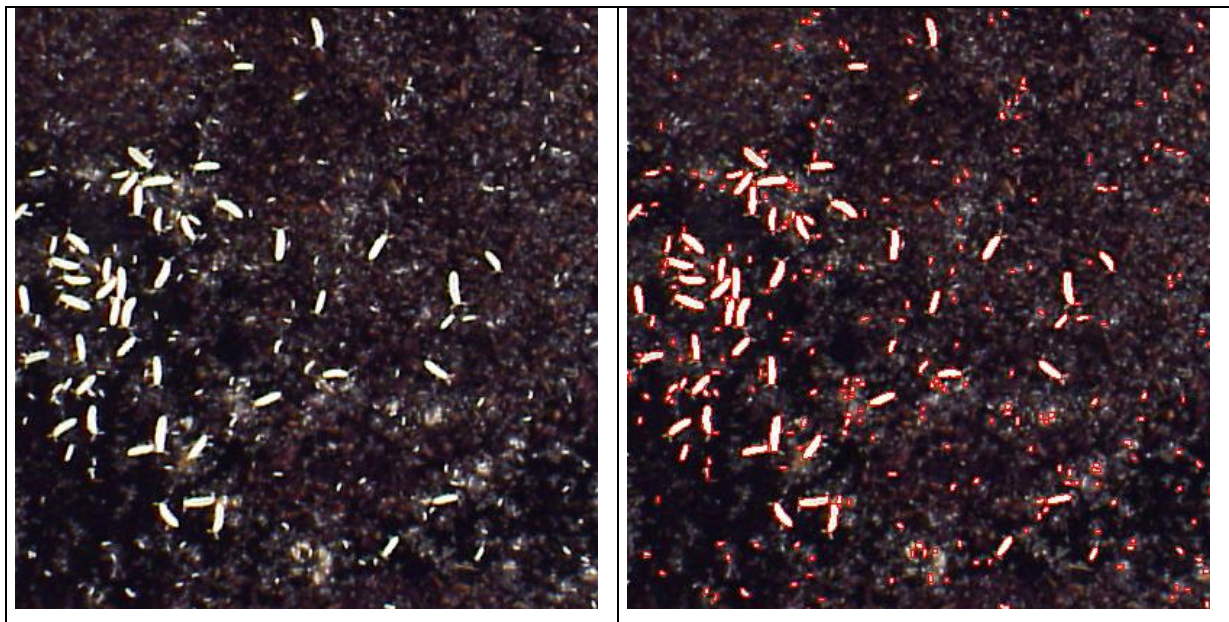
## Evaluation with the LemnaTec Scanalyzer

With the LemnaTec Scanalyzer the evaluation of the Collembola at the end of the is fast and reproducible and the Collembola software corresponds to the ISO-norm. The software is designed for a simple and efficient use in the laboratory practise. Special computer know-how or knowledge of image processing is not necessary.

Using the LemnaTec Scanalyzer guarantees constant recording conditions and high quality of the images. Therefore a high-resolution camera with a 1280 \* 960 pixel resolution is used. If the recording area is reduced the use of cameras with lower resolutions is possible.

With the high resolution, Collembola can be put in water in vessels up to a size between 10,5 \* 8 cm and then counted automatically. Using smaller vessels the resolution is increased and therefore the differentiation of size distribution is improved.

The illumination of the Scanalyzer is especially designed to ensure constant illumination conditions, to minimize reflections and make the digital images manageable for the image analysis.



**Original image (cutaway of vessels with the size of 10.5 \* 8cm) and assessment image**

With the highly developed image processing software from LemnaTec, even closed to each other laying Collembola, yeast, foam and floating soil components can be separated from each other and the Collembola can be quantified reproducibly. For the effective detection of smallest Collembola it is recommended to minimize yeast and foam of the samples.

Validation tests confirm a variance of the automatic evaluation from the visual counted value below 10%. For this reason the automatic evaluation is reliably in the range of the ISO norm standard.

All image analysis results are shown in the image. This allows an efficient quality control corresponding to GLP. Disturbing objects that are not detachable by image analysis (occasional yeast particles) can be removed manually within the scope of the quality control and the correction. Correction and visual control of the results are logged automatically. The user can adjust image analysis parameters to special conditions without having image processing know-how. If there are questions, LemnaTec makes evaluation methods available or optimises methods on the basis of the customer's images.

## **Data management**

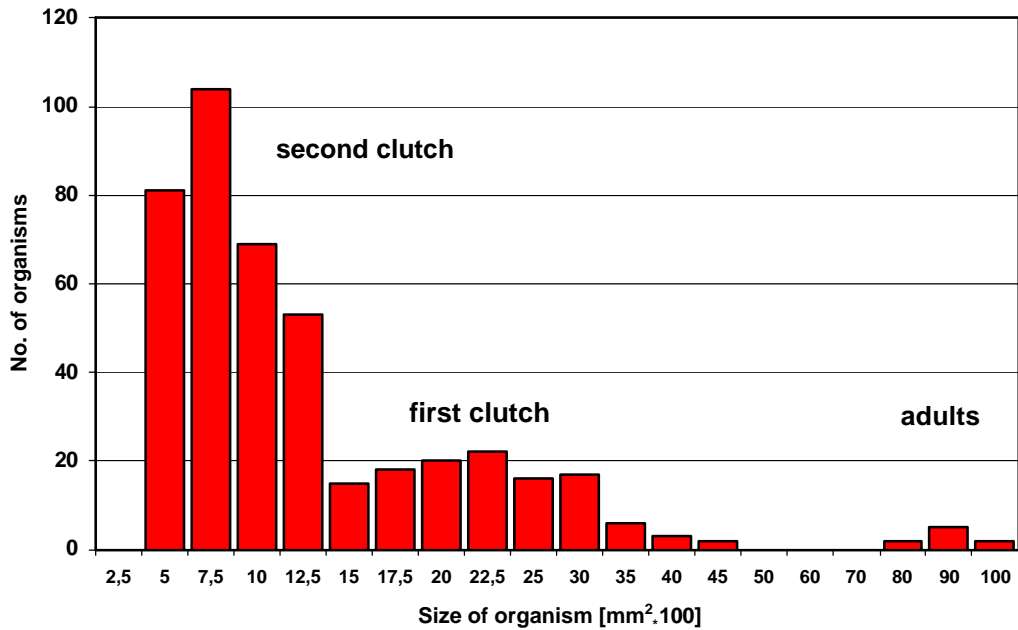
In addition to single images, measurement series can be entered after defining or loading a specification of the concentration levels, replicate numbers and the used concentrations. For the further facilitation and prevention of confusion a barcode scanner is available. After the termination of the image recording all images of the test are analysed automatically corresponding to the given methods. All data of the image recording, analysis and the results of measurement series are saved clearly and in compliance with GLP. All images, raw- and analysed data are available and can be exported to common spreadsheet programs.

For the evaluation of the EC-values the statistic module BioStat is available. With the help of this GLP-conform analysis module EC-values and confidence intervals are calculated and put out as tables and graphics.

In addition to the number of animals, the LemnaTec Scanalyzer detects automatically the size distribution of the Collembola. Thus the number of retrieved adult animals and the number of young animals can be detected corresponding to the norm specifications. Furthermore the Scanalyzer opens up the access to the size distribution, an important population quality. The size of the animals gives a statement on the fitness of the population and their reproduction dynamic.

This eases the objective and reproducible detection “of each pathologic or other symptom”, as required by the standard. The size distribution accesses the respective number and size of the animals from the first and the second clutch and creates a new ecotoxicological end point (Krogh 1998).

From the measurement of the size of all animals a total biomass parameter is derived. This provides the opportunity to use the total biomass parameter, in addition to the number, as additional test endpoint for the calculation of the inhibition values (here as summation of all visible Collembola areas). If the EC-value of the total collembola area differs significantly from the number, this is a significant indicator on secondary effects of the pollutant, that are not represented by the number.



**Size distribution of a control population. In addition to the adult animals, young collembola from two following clutches can be detected.**

Accurate tests for the formed biomass and for the organism's size are also very important for higher differentiated growth- and toxicity models, as e.g. the DebTox model (Nisbet 2000, Kooijman 2001).

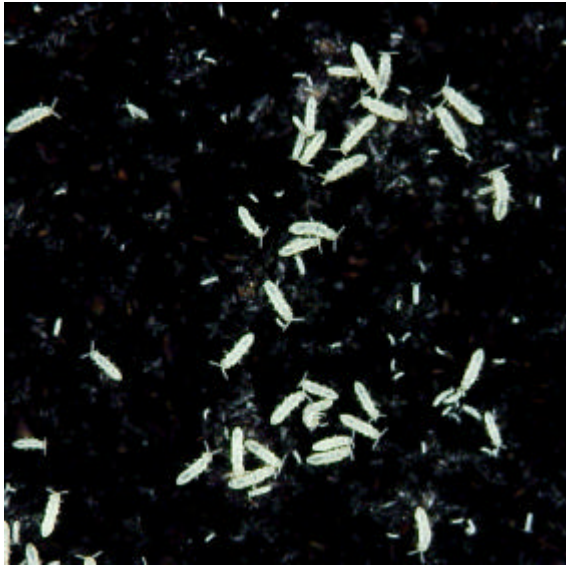
Other tests showed that a drift of the size distribution under substance influence and an associated reduction of the total biomass is an additional important toxicity parameter. This parameter is often more sensitive than the indistinctive count of all organisms (Jenner 1989, LemnaTec 2000, Hammers-Wirtz 2000).

For this reason the image analytical evaluation of the Collembolatest with the LemnaTec Scanalyzer allows an extensive quantification of effects in the retest for research purposes as well as for the processing of standardized tests.

### **Special implementations with high resolution**

Multirecordings of a large area-capturing unit can be performed and analysed as screening tests by shifting the vessel. The resolution of the images of single

collembola can be increased by the decrease of the area of interest or by a simple change of the lens. This can be necessary for special tests.



**Example cutouts from images with higher resolution (left: 50\*40 mm total image size , right 30\*25 mm total image size).**

### Literature:

**Fraunhofer (2001a)**, Ökologische und genotoxikologische Beurteilung von Böden und Substraten – Ringtestergebnisse Erich Schmidt Verlag

**Fraunhofer (2001b)**, Endbericht zum Verbundvorhaben “Validierung und praxisnahe Erprobung biologischer und ökotoxikologischer Tests zur Beurteilung belasteter und sanierter Böden und Substrate” IUCT Schmallenberg

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**ISO (1999)**, Soil Quality – inhibition of reproduction of collembola (*Folsomia candida*) by soil pollutants, ISO 11267:1999(E)

**Jenner HA, JPM Janssen-Mommen (1989)**, Phytomonitoring of pulverized fuel ash leachates by the duckweed *Lemna minor*, *Hydrobiologica* 188:189, 361-369

**Kooijman SALM (2001)**, Quantitative aspects of metabolic organization: a discussion of concepts *Phil. Trans. R. Soc. Lons. B*, 356, 331-349

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