# Soil improvers and growing media — Determination of plant response

Part 2: Petri dish test using cress

#### National foreword

This British Standard is the UK implementation of EN 16086-2:2011.

BSI, as a member of CEN, is obliged to publish EN 16086-2:2011 as a British Standard. However, attention is drawn to the fact that the UK committee voted against its approval as a European Standard.

The UK committee voted against the publication of this standard because, although the method is interesting for screening materials, the plant species specified for the test are not sensitive to some of the pesticides of greatest concern currently in composted materials, and because it considered that the reproducibility obtained in interlaboratory evaluation was not an adequate basis on which to establish a reference method. This could cause problems in the event of dispute or litigation.

The standard deviations of reproducibility in the inter-laboratory validation trials of this method were poor and there is a low probability of getting the same result from two laboratories analysing the same sample. In the worst cases the standard deviation of reproducibility exceeded the mean analysis (after rejection of outliers), for example mean germination rate for compost 1 was 22.38 %, S<sub>R</sub> 34.66; root length compost 1 0.98, S<sub>R</sub> 1.51 cm.

The UK committee advises that this standard will not detect inhibition by residues of some of the herbicides of greatest concern, and that in addition the results are imprecise.

The UK participation in its preparation was entrusted to Technical Committee AW/20, Top soil and other growing media.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Compliance with a British Standard cannot confer immunity from legal obligations.

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# **English Version**

# Soil improvers and growing media - Determination of plant response - Part 2: Petri dish test using cress

Amendements du sol et supports de culture -Détermination de la réponse des plantes - Partie 2: Essai en boîte de Pétri avec du cresson Bodenverbesserungsmittel und Kultursubstrate -Bestimmung der Pflanzenverträglichkeit - Teil 2: Petrischalentest mit Kresse

This European Standard was approved by CEN on 17 September 2011.

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Management Centre: Avenue Marnix 17, B-1000 Brussels

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# **Foreword**

This document has been prepared by Technical Committee CEN/TC "Soil improvers and growing media", the secretariat of which is held by ASI.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2012, and conflicting national standards shall be withdrawn at the latest by May 2012.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

SAFETY PRECAUTIONS – Care should be taken when handling samples that may contain sharps or is of a dusty nature.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

# 1 Scope

This European Standard describes a method for the routine determination of the effect of soil improvers and growing media or constituents thereof on the germination and early root development of cress.

#### 2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 13037, Soil improvers and growing media – Determination of pH

EN 13038, Soil improvers and growing media – Determination of electrical conductivity

EN 13040, Soil improvers and growing media – Sample preparation for chemical and physical tests, determination of dry matter content, moisture content and laboratory compacted bulk density

EN ISO 3696, Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

#### 3.1

#### plant response

variation in cress seed germination and/or growth when sown and grown in a growing medium, soil improver or constituent thereof

NOTE Factors causing negative plant growth cannot be identified nor sufficiently quantified by applying this method.

#### 3.2

## germination

for this method, the seed is said to have germinated as soon as the radicle has emerged from the seed

#### 3.3

## root length index

percentage difference of the root length of germinated cress seeds on the material under investigation compared to the root length of the control

#### 3.4

# Munoo-Liisa Vitality index

index calculated from the germination rate and the root length

# 4 Principle

Cress seeds are exposed to the test material for a few days under controlled conditions. The germination and growth of young roots are measured and compared with a control sample.

The inhibition of germination and growth of young roots may be caused by phytotoxic substances. If the electrical conductivity (EC) in the diluted material is greater than 80 mS m<sup>-1</sup> according to EN 13038, the sample is diluted with sphagnum peat. In this case, the test does not measure the adverse effect of high EC of materials on germination and root development.

NOTE 1 In the case of composted materials, these phytotoxic substances can be for instance ammonia, ethylene oxide or short chain fatty acids.

NOTE 2 The test can also be used as an indication of the instability and "immaturity" of the material.

If required (for example to fulfil certain quality certification requirements or legislation), materials can be tested without checking the EC.

For testing of specific effects, the use of additional plant species such as Chinese cabbage or lettuce may be considered.

# 5 Choice of methodology

#### 5.1 Contact method

For most of growing media or soil improvers, the petri dish test can be carried out with the seeds in physical contact with the material to be tested.

#### 5.2 Extract method

Coarse samples such as bark, expanded clay, lava, mineral wool, perlite, polyurethane and pumice, used at 100 % as a growing medium, are not suitable for this procedure. For these materials, the seeds should be in contact with a filter paper thoroughly wetted with an extract of the material to be tested.

# 6 Material

# 6.1 Cress seeds (Lepidium sativum)

Germination capacity ≥ 95 %.

#### 6.2 Water of class 3

According to EN ISO 3696.

# 6.3 Sphagnum peat

Sphagnum peat with a degree of humification H3 - H5 according to von Post scale, a pH between 3,0 and 4,5 (measured according to EN 13037), an EC of between 1 and 5 mS m<sup>-1</sup> (measured according to EN 13038) and a particle size < 10 mm; without pH-adjustment or fertilizer addition.

## 6.4 Fertilized and limed Sphagnum peat

Sphagnum peat (see 6.3), pH-adjusted using ground limestone to a range between 5,5 and 6,5 measured according to EN 13037, fertilized with a water soluble complete fertilizer with essential micronutrients, supplying (225 ± 25) mg N ·  $\Gamma^1$  (for example 1,5 g ·  $\Gamma^1$  water soluble complete fertilizer N :  $P_2O_5$ :  $K_2O_7 = 15 : 10 : 20$ ).

NOTE See Annex B.2.

#### 6.5 Petri dishes

Square, nominal 100 mm length and width, nominal 18 mm height.

#### 6.6 Perlite

Particle size < 2,5 mm, maximum 20 % W/W < 0,5 mm.

# 6.7 Testing facility

Temperature controlled room or growth chamber which can be set at (25 ± 5) °C.

### 6.8 Sieve with 10 mm mesh size

# 6.9 Filter paper

Approximately 1,42 mm thickness, approximately 700g/m<sup>2</sup> weight (for example Whatman "blotter light blue 3644" or equivalent product).

#### 6.10 Ground limestone

Finely ground limestone, containing at least 5 % MgCO<sub>3</sub>, having a particle size less than 1 mm and a moisture content of less than 1 % m/m.

#### 7 Contact method

# 7.1 General preparation

Pass the sample through a 10 mm sieve (see 6.8). Any foreign material such as plastic, metal or glass retained on the sieve shall be removed, the percentage shall be noted. Any other material retained on the sieve which is an intrinsic part of the sample shall be physically reduced to parts of similar size as few times as are necessary to permit the entire sample to pass through the sieve. Fibrous materials i.e. coir fibres and straw shall be cut to a length  $\leq$  10 mm by using scissors. Thoroughly mix the laboratory sample with the broken particles retained on the sieve taking care to minimise physical damage to the sample as a whole. Transportation and possible storage of the samples shall be done in accordance with EN 13040, using food grade polyethylene bags.

NOTE In cases where the proportion of the retained material is above 30 % weight, the extract method is more appropriate.

#### 7.2 Sample storage and preparation

If necessary, samples can be stored according to EN 13040. The material to be tested shall be moistened to the approximate optimum moisture content according to the fist test (see Annex B).

The electrical conductivity of the moistened test sample shall be determined according to EN 13038. If the EC of the sample is > 80 mS m<sup>-1</sup>, the sample shall be diluted with a sufficient amount of sphagnum peat (see 6.3) until the EC does not exceed 80 mS m<sup>-1</sup>. The pH according to EN 13037 is ideally within the range between 5,5 and 6,5. If it is below, the pH shall be adjusted by adding limestone (see 6.10). After adding limestone, the sample shall be equilibrated for 24 h.

NOTE Usually, 2 g to 3 g of limestone per litre should be sufficient.

If required (for example to fulfil certain quality certification requirements or legislation), materials can be tested without checking the EC.

#### 7.3 Procedure

Fill the petri dish completely and level the surface (for example with a spatula) without heavy compression. Where the seed is being placed, remove any particles > 5 mm. Sow 10 cress seeds per dish evenly spaced on the test material 10 mm to 20 mm from the top and press the seed gently into the surface of the test material. It is important that there is good contact with the test material. To ensure this, a drop of water shall be added to each seed using a pipette. Perform the procedure in at least three replicates.

NOTE 1 A higher number of replicates can be used. The number of replicates should be taken into account for the calculation of the results.

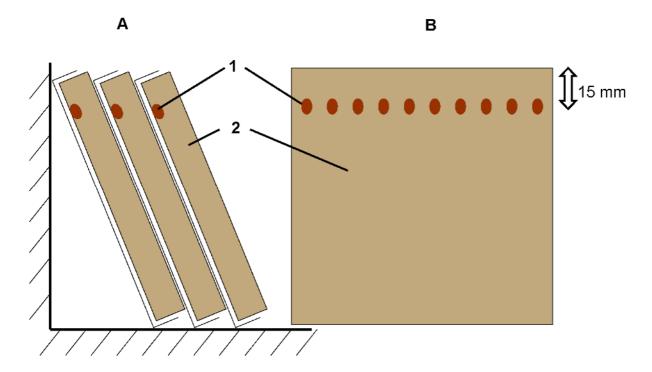
As a control sample, perform the same procedure with limed and fertilized sphagnum peat (see 6.4), in three replicates.

NOTE 2 As a "positive" reference, the procedure can be performed using fertilized Sphagnum peat (see 6.4) wetted with a solution of acetic acid resulting in a final concentration of approximately 350 mg acetic acid per litre of sphagnum peat. This should give a reduction of the germination rate and/or the root length of about 50 %.

Close the dishes with their covers and incubate with the Petri dish placed  $70^{\circ}$ to  $80^{\circ}$  to the horizontal with the end where the seeds are placed uppermost and with the substrate on the lower surface in the dark at  $(25 \pm 5)$  °C (see Figure 1). Incubate as described for 72 h. Determine the percentage germination (germination rate) root development (root score) by measuring the length in mm. If the average germination rate (see 7.4) in the reference material is below 85 %, the test is invalid.

NOTE 3 The covers can be fixed by using a rubber band or wrapping them in aluminium foil. If the Petri dish is completely covered by the foil, it can be incubated without additional darkening.

NOTE 4 A digital photograph can be taken and an image analysis can be prepared using an image analysis programme. This will give root length and root diameter and the results can be reported as percentage of control.



# Key

A side view

B front view

1 cress seed

2 substrate/perlite

Figure 1 — Petri dish placement for incubation

Roots can be stored in bottles containing 50 % V/V ethanol at (5 ± 3) °C and measured later.

# 7.4 Evaluation parameters

Calculate the coefficient of variance for the germination rate and the root length for the test sample and the control according to Equations (1), (2), (3), (4) and (5).

$$AGR = \frac{GR(\operatorname{dish} 1) + GR(\operatorname{dish} 2) + GR(\operatorname{dish} 3)}{3} \tag{1}$$

where

AGR is the average germination rate;

*GR* is the germination rate.

$$CVG = \frac{\sqrt{\frac{\sum (GR - AGR)^2}{2}}}{\frac{2}{AGR}} \cdot 100 \tag{2}$$

where

CVG is the coefficient of variance for the germination rate.

$$RLP = \frac{\sum RL}{NGS} \tag{3}$$

where

*RLP* is the root length, per plant;

RL is the root length;

*NGS* is the number of germinated seed.

$$ARLP = \frac{RLP(\text{dish 1}) + RLP(\text{dish 2}) + RLP(\text{dish 3})}{3}$$
(4)

where

ARLP is the average root length, per plant.

$$CVR = \frac{\sqrt{\frac{\sum (RLP - ARLP)^2}{2}}}{\frac{2}{ARLP}} \cdot 100 \tag{5}$$

where

CVR is the coefficient of variance for the root length.

The root **length** index (RI) is expressed as a percentage difference of the root length of germinated cress seeds on the tested material per dish compared to the average root length of all the control samples and is calculated according to Equation (6)

$$RI(\%) = \frac{\left(\frac{RL_{s1}}{RL_c} + \frac{RL_{s2}}{RL_c} + \frac{RL_{s3}}{RL_c}\right)}{3} \cdot 100$$
 (6)

where

*RI* is the root length index;

 $RL_{s1}$  is the average root length of first replicate;

 $RL_{s2}$  is the average root length of second replicate;

 $RL_{s3}$  is the average root length of third replicate;

 $RL_c$  is the average root length of the control samples.

The Munoo – Liisa vitality index (MLV) compares the product of germination of seeds in the tested material (%) and the average root length in the test and control samples and is calculated according to Equation (7).

$$MLV (\%) = \left(\frac{\left(GR_{s1} \cdot RL_{s1}\right) + \left(GR_{s2} \cdot RL_{s2}\right) + \left(GR_{s3} \cdot RL_{s3}\right)}{3 \cdot \left(GR_{c} \cdot RL_{c}\right)}\right) \cdot 100 \tag{7}$$

where

MLV is the Munoo – Liisa vitality index of the sample (% compared with control);

 $GR_{s1}$  is the germination rate in first replicate, in %;

 $GR_{s2}$  is the germination rate in second replicate, in %;

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 $GR_{s3}$  is the germination rate in third replicate, in %;

 $GR_c$  is the average germination rate of the control samples, in %;

 $RL_{s1}$  is the average root length of first replicate;

 $RL_{2}$  is the average root length of second replicate;

 $RL_{s3}$  is the average root length of third replicate;

 $RL_c$  is the average root length of the control samples.

#### 8 Extract method

# 8.1 Preparation of the sample extract

Fill a watertight container of approximately 1 litre volume to the rim with material to be tested and compact by dropping 3 times from  $(3 \pm 2)$  cm height.

Add nutrient solution (see B.2) slowly until the material is just covered. If the material floats on the liquid, cover the top with a mesh. Leave for 4 h at ambient temperature. Pour out and retain the resultant liquid extract and supplement this with liquid pressed from the residual medium by hand. Include a remark in the report stating that the extraction method is used.

The electrical conductivity of the extract shall be measured according to EN 13038. If the EC of the extract is  $> 250 \text{ mS} \cdot \text{m}^{-1}$ , the sample shall be diluted with a sufficient amount of water (see 6.2) until the EC does not exceed 250 mS·m<sup>-1</sup>. If the EC is below 250 mS m<sup>-1</sup>, record the actual value.

The extract shall be used as rapidly as possible after preparation.

If required (for example to fulfil certain quality certification requirements or legislation) materials can be tested without checking the EC.

#### 8.2 Procedure

Fill the petri dish with perlite (see 6.6) and level the surface (for example with a spatula). Place a sheet of filter paper (see 6.9) cut to fit the Petri dish on top of the perlite and wet it evenly with  $(50 \pm 2)$  ml of the extract.

NOTE 1 The seed testing paper should completely absorb this added extract.

Sow 10 cress seeds (see 6.1) per dish evenly spaced on the filter paper 10 mm to 20 mm from the top. It is important that there is good contact with the filter paper. To ensure this, a drop of water shall be added to each seed using a pipette. Perform the procedure in at least three replicates.

As a control sample, perform the same procedure using a nutrient solution as described in Annex B instead of the extract.

Close the dishes with their covers and incubate with the Petri dish placed  $70^{\circ}$  to  $80^{\circ}$  to the horizontal with the end where the seeds are placed uppermost and with the substrate on the lower surface in the dark at  $(25 \pm 5)$  °C (see Figure 1). Incubate as described above for 72 h. Determine the percentage germination (germination rate) and root development (root score) by measuring the length in mm. If the average germination rate (see 7.4) in the reference material is below 85 %, the test is invalid.

NOTE 2 The covers can be fixed by using a rubber band or wrapping them in aluminium foil. If the Petri dish is completely covered by the foil, it can be incubated without additional darkening.

NOTE 3 A digital photograph can be taken and an image analysis can be prepared using an image analysis programme. This will give root length and root diameter and the results can be reported as percentage of control.

Roots can be stored in bottles containing 50 % V/V ethanol at (5 ± 3) °C and measured later.

# 8.3 Evaluation parameters

Determine and calculate the evaluation parameters according to 7.4.

# 9 Test report

The test report shall include the following information:

- a reference to this European Standard;
- all data required for a complete identification of the sample;
- test with or without EC-check;
- dilution ratio used to reduce EC;
- deviation of EC in the extract (if using extraction method);
- the average germination rate and its coefficient of variance of the sample;
- the average germination rate and its coefficient of variance of the control;
- the average root length and its coefficient of variance of the sample;
- the average root length and its coefficient of variance of the control;
- the root length index;
- the ML vitality index;
- details of all work cycles not contained in this standard or that were considered optional, as well as all factors that may have influenced the results.

# Annex A (informative)

# **Validation**

# Samples:

PBGM 1 (I): peat based growing medium no. 1, contact method

PBGM 1 (II): peat based growing medium no. 1, extract method

PBGM 2: peat based growing medium no. 2, contact method

Bark (I): uncomposted bark, contact method

Bark (II): uncomposted bark, extract method

Compost 1: compost no. 1, contact method

Compost 2: compost no. 2, contact method

In Table A.1 to A.6, the statistical results of the interlaboratory test are given.

Table A.1 — Summary of the results of the Petri dish test – AGR (average germination rate)

Sample	PBGM 1	PBGM 1	PBGM 2	bark (I)	bark (II)	compost 1	compost 2
	(I)	(II)					
				%			
Number of laboratories retained after							
eliminating outliers	11	11	10	12	12	7	7
Number of outliers (laboratories)	0	0	0	0	1	0	0
Mean value	96,97	88,94	94,00	72,22	88,79	22,38	28,10
Repeatability standard deviation, $s_{\rm r}$	5,29	11,45	9,13	9,28	17,69	6,17	10,69
Repeatability relative standard deviation	0,06	0,13	0,10	0,13	0,20	0,28	0,38
Repeatibility limit, $r = 2.8 s_r$	14,82	32,06	25,56	25,98	49,53	17,28	29,93
Reproducibility standard deviation, $s_R$	0,00	16,12	1,96	29,24	11,09	34,66	36,85
Reproducibility relative standard deviation	0,00	0,18	0,02	0,41	0,13	1,55	1,31
Reproducibility limit, $r = 2.8 s_R$	0,00	45,13	5,48	81,86	31,05	97,04	103,17

Table A.2 — Summary of the results of the Petri dish test – ARLP

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I)	bark (II)	compost 1	compost 2
	cm						
Number of laboratories retained after							
eliminating outliers	11	11	10	12	12	7	7
Number of outliers (laboratories)	0	0	0	0	0	0	0
Mean value	4,52	3,89	3,94	2,69	3,80	0,98	1,58
Repeatability standard deviation, $s_r$	0,68	0,85	0,67	0,70	1,14	0,43	1,51
Repeatability relative standard deviation	0,15	0,22	0,17	0,26	0,30	0,44	0,96
Repeatibility limit, $r = 2.8 s_r$	1,91	2,37	1,89	1,96	3,20	1,22	4,22
Reproducibility standard deviation, $s_R$	1,09	0,84	0,89	1,71	0,97	1,51	1,25
Reproducibility relative standard deviation	0,24	0,22	0,23	0,64	0,25	1,54	0,79
Reproducibility limit, $r = 2.8 s_R$	3,04	2,34	2,50	4,78	2,70	4,24	3,50

Table A.3 — Summary of the results of the petri dish test – RI

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I)	bark (II)	compost 1	compost 2
	%						
Number of laboratories retained after							
eliminating outliers	11	11	10	12	11	6	7
Number of outliers (laboratories)	0	0	0	0	1	1	0
Mean value	96,21	96,86	84,30	56,73	93,51	7,91	30,80
Repeatability standard deviation, $s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability relative standard deviation	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatibility limit, $r = 2.8 s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Reproducibility standard deviation, $s_R$	27,16	5,50	24,77	44,79	20,29	7,47	15,26
Reproducibility relative standard deviation	0,28	0,06	0,29	0,79	0,22	0,95	0,50
Reproducibility limit, $r = 2.8 s_R$	76,05	15,40	69,36	125,40	56,81	20,92	42,73

Table A.4 — Summary of the results of the petri dish test – MLV

Sample	PBGM 1 (I)	PBGM 1 (II)	PBGM 2	bark (I)	bark (II)	compost 1	compost 2
	%						
Number of laboratories retained after							
eliminating outliers	11	11	10	12	12	6	6
Number of outliers (laboratories)	0	0	0	0	0	1	1
Mean value	97,65	91,22	83,25	46,02	87,58	3,01	2,99
Repeatability standard deviation, $s_{\rm r}$	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatability relative standard deviation	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Repeatibility limit, $r = 2.8 s_r$	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Reproducibility standard deviation, $s_R$	16,07	9,55	27,65	20,46	36,31	3,98	2,41
Reproducibility relative standard deviation	0,17	0,11	0,33	0,45	0,42	1,32	0,81
Reproducibility limit, $r = 2.8 s_R$	45,01	26,74	77,41	57,28	101,66	11,14	6,75

# Annex B (normative)

# Fist test, nutrient solution

## **B.1 Fist test**

The fist test shall be carried out wearing flexible protective gloves.

If storage is necessary, samples are kept in accordance with EN 13040. Prior to analysis, samples are prepared by sieving < 10 mm). The material to be tested shall be adjusted to a moisture content that is optimal for plant growth prior to testing using the following procedure:

The sample is pressed in the fist. If water beads escape between the fingers, the sample is too wet. If the sample crumbles in the hand when the fist is opened, without further action, the sample is too dry. Suitable moisture content is present if the pressed sample forms an aggregate which crumbles under mild pressure, after the fist has been opened; if, on the contrary, it only deforms, it is too wet. The optimal moisture content is that which may be described as "moist as a well-squeezed sponge". When moistening excessively dry sample material the water shall be mixed into the sample material in such a manner that it is evenly absorbed. In the case of very dry samples, this process requires thorough mixing at intervals. This procedure shall last no more than 8 h. Excessively moist samples shall be carefully air-dried (< 30 °C) and thoroughly mixed thereafter.

# **B.2** Composition of the nutrient solution

#### Reagents:

Ammonium nitrate,  $NH_4NO_3$  Calcium nitrate,  $Ca(NO_3)_2 \cdot 4 H_2O$  Potassium nitrate,  $KNO_3$  Mono-potassium phosphate,  $KH_2PO_4$  Magnesium sulphate,  $MgSO_4 \cdot 7 H_2O$  Magnesium nitrate,  $Mg(NO_3)_2 \cdot 6 H_2O$  Iron-chelate, Fe - DTPA (7% Fe) Manganese sulphate,  $MnSO_4 \cdot H_2O$  Zinc sulphate,  $ZnSO_4 \cdot 7 H_2O$  Borax,  $Na_2B_4O_7 \cdot 10 H_2O$  Copper sulphate,  $CuSO_4 \cdot 5 H_2O$  Sodium molybdate,  $Na_2MoO_4 \cdot 2 H_2O$ 

Nitric Acid (HNO<sub>3</sub>), 1M

Table B.1 shows the composition of the nutrient solution.

Table B.1 — Composition of the nutrient solution

Chemical species	mmol/l	Chemical species	μmol/l
NH <sub>4</sub> <sup>+</sup>	1,0	Fe	15
K <sup>+</sup>	8,0	Mn	8
Ca <sup>⁺</sup>	4,0	Zn	4
Mg <sup>2+</sup>	1,5	В	25
NO <sub>3</sub>	16,0	Cu	0,75
SO <sub>4</sub> <sup>2-</sup>	1,25	Мо	0,5
H <sub>2</sub> PO <sub>4</sub>	1,5		

For preparing one litre of nutrient solution (Table B.1), the following quantities of chemicals (p.a.) have to be dissolved in water.

Table B.2 — Amounts of chemicals to be dissolved

Name	Chemical compound	mmol/l	mg/l
Ammonium nitrate	NH <sub>4</sub> NO <sub>3</sub>	1,0	80
Calcium nitrate	Ca(NO <sub>3</sub> ) <sub>2</sub> · 4 H <sub>2</sub> O	4,0	944
Potassium nitrate	KNO <sub>3</sub>	6,5	657
Mono-potassium phosphate	KH <sub>2</sub> PO <sub>4</sub>	1,5	204
Magnesium sulphate	MgSO <sub>4</sub> · 7 H <sub>2</sub> O	1,25	308
Magnesium nitrate	$Mg(NO_3)_2 \cdot 6 H_2O$	0,25	65
Name	Chemical compound	μmol/l	μg/l
Iron-chelate	Fe-DTPA (7% Fe)	15	11,97
Manganese sulphate	MnSO <sub>4</sub> · H <sub>2</sub> O	8	1,35
Zinc sulphate	ZnSO <sub>4</sub> · 7 H <sub>2</sub> O	4	1,15
Borax	Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> · 10 H <sub>2</sub> O	6,26	2,39
Copper sulphate	CuSO <sub>4</sub> · 5 H <sub>2</sub> O	0,75	0,19
Sodium molybdate	Na <sub>2</sub> MoO <sub>4</sub> · 2 H <sub>2</sub> O	0,5	0,12

NOTE Stirring and warming may be necessary to dissolve the components completely.

Adjust the pH of the solution to reach 5,5, using 1M nitric acid.

All chemicals used have to be of analytical grade.

Alternatively, commercially available fertilizers of similar composition dissolved in water can be used to provide the same concentration of prescribed elements, especially nitrogen. The pH-adjustment is necessary in any case.

The same chemicals/products should be used for both the sample and the limed and fertilized sphagnum peat.

# **Bibliography**

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Zucconi F,Pera A, Forte M and M De Bertoldi 1981. *Evaluating toxicity of immature compost.* Biocycle 22(2) 54-57.