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Phytotoxicity *(Plant tolerance)*

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY

The assessment of phytotoxicity is one of the major criteria if using soil, sludge or composted biowaste as well as any kind of plant substrate (growing media) and soil improvers. One task of this desk study was to give a survey over the existing standards and methods. A difficulty lies in the fact, that there are only few finished standards. Furthermore the horizontal method shall be applicable on quite different materials and the test results will be used for different purposes. For testing, plants are either grown in the material directly or on inert substrates provided with leachates of the tested material. For this draft, the first approach has been chosen. A variety of test plants is available. The individual choice of the test plant can be made according to the scope of the test. In any case, at least one monocotyledonous and one dicotyledonous species has to be used.

1. INTRODUCTION

For the use of soil, sludge, biowaste compost, soil improvers, growing media and any kind of plant substrate toxic effects towards the environment have to be avoided. The organisms most likely to suffer immediately and visibly from adverse effects are higher plants. Therefore, without a detailed specification of the cause, the phytotoxic effect of a certain material can be used as an indicator.

Phytotoxicity is defined as a delay of seed germination, inhibition of plant growth or any adverse effect on plants caused by specific substances (phytotoxins) or growing conditions (PAS 100). If a phytotoxic effect of a certain material is stated, further investigations should be carried out to identify the specific cause.

There is a wide range of methods for examining phytotoxicity, although there are hardly any national standards. Therefore, various key parameters of these methods, e.g. the test substances the test plants, the individual procedures and test parameters differ to a large extent (mostly depending on the material under investigation). Some of these bioassays (e.g. ASTM methods, OECD, 1984, U.S. EPA, 1985) have been already evaluated and compared in reviews (Keddy et al., 1995, Kristen, 1997, Calow, 1997, Kapustka**,** 1997, GCPF, 2000).

In this desk study we

- identified the key parameters of the different methods for the substrates cited above
- listed the differences and similarities between the test procedures for the most important parameters
- tried to explain the choices when writing the draft of the horizontal standard

The proposed draft method is designed to create an optimum version by combining the most suitable parts of different test procedures.

In this second issue of the desk study, the comments given up to now have been included as far as possible.

2. EXISTING METHODOLOGY

2.1 Standards or draft standards

ASTM: Method E1598-94: Practice for Conducting Early Seedling Growth Tests (1994).
CEN/TC 223 (2003-07): Growing media-Biotest for assessment of phytotoxicity.

ISO 11269-1: Soil Quality – Determination of the effects of pollutants on soil flora – Method for the Measurement of Inhibition of Root Growth (1993)

ISO 11269-2:1995 Soil Quality – Determination of the effects of pollutants on soil flora – Effects of chemicals on the emergence and growth of higher plants

ISO/CD 17126 Soil Quality – Determination of the effects of pollutants on soil flora – Seedling emergence, screening test with lettuce (Lactuca sativa (L.))

OECD-Guideline for the testing of chemicals: Proposal for updating Guideline 208, 2000

ÖNORM S 2021 (draft) 2004-03-01: Growing media - Quality Requirements and test methods

VDLUFA-Methodenbuch (1997): Nachweis von pflanzenschädigenden Stoffen in Böden, gärtnerischen Substraten und Komposten.
VDLUFA-Methodenbuch (1997): Nachweis von gasförmigen pflanzenschädigenden Stoffen in Böden, gärtnerischen Substraten und Komposten

2.2 Other than standard methods

The following methods have been supplied by organisations other than standardisation bodies:

WRAP, The Composting Association (2002): Public Available Specification 100 – Specification for composted material, Annex D: Method to assess contamination by weed propagules and phytotoxins in composted material

Federal Compost Quality Assurance Association, Germany (1998): Determination and evaluation of phytotoxicity of compost by means of a germinating plant test with summer barley

Fuchs, J.G. (2000): New biotests to measure the biological qualities of compost. AgrarForschung 7(7): 314 – 319

Petersen, L.: Water Extract – a new method for a bioassay; DEG Green Team, Denmark

Rijkslaboratorium Gent: Phytotoxicity

KIWA: Test of Phytotoxicity

RHP-foundation: Phytotoxicity

2.3 National law:

2.3.1.1.1 Austrian compost Ordinance

German Ordinance on the utilization of Bio-Wastes on land used for agricultural, silvicultural and horticultural purposes

2.4 Overview of selected methods for the assessment of phytotoxicity on higher plants

Table 1 Standards or draft standards – a survey

Number	Title	Principle	Test plant	Test material	Reference material	Test parameters
ISO 11269-2:1995	Soil Quality – Determination of the effects of pollutants on soil flora – Effects of chemicals on the emergence and growth of higher plants	Emergence and early growth response to chemicals added to the test soil	-Rye -ryegrass, perennial -rice -oat -wheat, soft -barley -Sorghum -Sweetcorn -mustard -rape -radish -turnip -chinese cabbage -birds foot fenugreek -Lettuce -Cress, garden -Tomato -Bean	Test Soil (sterile or non sterile) with solid or liquid chemicals incorporated	Test soil	For each replicate: -Number of seedlings emerging -Number of plants remaining at harvest -Total mass (fresh or dry) at harvest
ISO/CD 17126	Soil Quality – Determination of the effects of pollutants on soil flora – Seedling emergence, screening test with lettuce (Lactuca sativa (L.)	Procedure for the determination of effects of contaminated soil or other contaminated samples (soil materials, compost, sludge) on the emergence of lettuce seeds. They are exposed to the test material under investigation in a geometric dilution series with test material and growth medium.	- lettuce seeds (Lactuca sativa (L.)	soils, soil materials, compost, sludge; Not dried, sieved (<2mm) **test material** (soil or other) with registered water content, water holding capacity, EC, and pH. **Chemicals** are tested with adding them – dissolved in water or organic solvent - to the growth medium.	Growth medium: washed, fine quartz sand, e.g. with grain size 0.4-0.8mm; (Cover material: washed coarser quartz sand e.g. with grain size 0.7-1.2 mm (possibly 0.8-1.4mm))	Results: - number of seedlings emerged, - EC 50 (EC 20): 50% (80%) of the mean seedling emergence - mean seedling emergence in the controls

Number	Title	Principle	Test plant	Test material	Reference material	Test parameters
OECD 208	OECD GUIDELINE FOR THE TESTING OF CHEMICALS PROPOSAL FOR UPDATING GUIDELINE 208 Terrestrial (Non-Target) Plant Test 208 A: Seedling Emergence and Seedling Growth Test DRAFT DOCUMENT July 2000	Assessment of the seedling Emergence and seedling Growth of higher plants following the exposure to the test substance in the soil (or other suitable matrix). Assessment of the phytotoxicity of solid and liquid substances (general chemicals and crop protection products, CCP)	For chemicals three species (one monocot. and two dicot. representing three families, for crop protection products 6-10 species representing 2 monoc. and 4-6 dicot. families; mono:di:1:2; From a list in Annex 2)	Test substance applied in potted soil (sandy loam, l. sand, l. clay or clay loam (commercial potting soil or synthetic soil mixes- not clay.) Field soil < 2mm, soil type, texture 5. Glass beads, mineral wool, acid washed sand is not recommended for testing CPP, for chemicals it is possible	Untreated test material (soil or artificial substrate)	Results: - number and % emergence as compared to the controls; - biomass measurements (i.e. shoot weight - fresh or dry-, or shoot height as a % of the controls) -% visual injury and description of the rating scale used to judge it -description of the statistical procedures, etc. Statistical analysis: single rate test, multiple rate test. Test report: detailed description of the - test substance - test species - test conditions - results
CEN/TC 223 (working document)	Growing media- Biotest for assessment of phytotoxicity	Evaluation of the phytotoxicity of a growing medium towards the growth of seedlings in representative conditions of the habitual use of a growing medium. Substrate is evaluated in controlled conditions close to normal conditions of cultivation, **after seedlings transplanting**, during the first pahses of growth.	Lettuce (*Latuca sativa*), petunia *(Petunia hyb.)*, tomato (*Lycopersicum esculentum)*, geranium *(Pelargonium zonale)* or impatiens *(Impatiens hyb.)* Seedlings are obtained in small cells, their content is the lump of transplanting	Growing medium, limed and fertilised so as to reach a conductivity, pH and conc. In N, P_2O_5, K_2O close to reference; Sieved<10mm (20mm)	A blend of perlite (20%) and white peat (80%) with fine granulometry (0-8mm); pH: 5.5-7.5; N, P_2O_5, K_2O *(1:0.5:1.4)*;conductivity 25-50mS.m^{-1} (extr. 1/5, v/v)	- Dry biomass - qualitative can be made: leaves aspect, count of flowers per plant
XP U 44-167 (French standardization – working document)	Organic soil improvers - Biotest for the assessment of phytotoxicity in conditions of use	Assessment of the possible phytotoxicity of organic soil improvers (organic fertilisers) by measuring their effects on the emergence and growth orf higher plants. The aim is to show that the dosage recommended is not toxic to plants	- Rice - Oats - Wheat - Barley - Radix - Lettuce - Garden cress - Tomato - Red Kidney bean	Soil with added Soil improver at different concentrations	Mixture of standard soil and perlite 80:20vol%	-number of emerged seeds - aerial biomass (moist or dry) for each repetiotion

Number	Title	Principle	Test plant	Test material	Reference material	Test parameters
ÖNORM S 2021 (draft)	Quality Requirements and test methods for growing media	Phytotoxicity – cress test	-Cress - *Lepidium sativum* (also - timothy – *phleum pratense*, -chinese Cabbage - *Brassica campestris L. var. chinensis*, - spring barley *(hordeum vulgare))*	-Growing media -compost	mixture of commercially available substrate, low in nutrient content (e.g. for germination), with burnt flour of clay (1:1 mass)	-Mean fresh mass (at least 3 replicates), additionally: -germination rate in % compared to the standard substrate -delay of germination in days compared to the standard substrate - colour of the plants compared to the standard -abnormality of plants and plant growth
VDLUFA-Methodenbuch (1997)	Nachweis von pflanzenschädigenden Stoffen in Böden, gärtnerischen Substraten und Komposten Evidence of phytotoxic substances in soils, horticultural substrate and composts	Detection of pollutants (uptake with the root) in soils, products of bark, compost, growing media). Comparison of the seedling emergence and –growth on the test substrate compared with the reference material	Chinese Cabbage – *Brassica napus var. chinensis*, -spring barley *(hordeum vulgare))*	soils, products of bark, compost, growing media	-For mineral soils: arable soil, medium textured, pH: 6-6.5, org. matter > 1.5-2% -for peat soils: standard substrate (also for the dilution of mixtures of compost)	-yields (fresh and dry mass) -germination rate -valuation (development of cotyledons, colour, root health and –intensity, plant development)
VDLUFA-Methodenbuch (1997)	Nachweis von gasförmigen pflanzenschädigenden Stoffen in Böden, gärtnerischen Substraten und Komposten Evidence of gaseous phytotoxic substances in soils, horticultural substrate and composts	Placement of the scrutinising substance in a preserving glass, sowing of cress on it, placement of a moist piece of cotton wool with cress seed on it into the glass without contact to the substance, placement of the covered glass in a germination facility ; observation of the plant development on the cotton wool, valuation of the emergence	Cress	soils, horticultural substrates, products of bark, compost and raw material for substrates that have an phytotoxic effect after transition into the gas phase	-arable soil, medium textured, pH: 6-6.5, org. matter > 1.5-2% - standard substrate for peat soils	Daily rating of the radicles and - if necessary- damages of the cotyledons on the cotton wool and on the test substance according to a scheme

Table 2: Other than standard methods - a survey:

Number	Title	Principle	Test plant	Test material	Reference material	Test parameters
WRAP, The Composting Association (2002)	Public Available Specification 100 – Specification for composted material, Annex D: A Method to assess phytotoxins in composted organic material	The response of indicator plants to phytotoxins is determined using an amended sample under controlled growing conditions	**Lettuce**, Winter density **Radish**, French breakfast **Lentil** (as available from supermarkets)	Diluted (with vermiculite) Composted materials (not necessarily for all types of compost)	-Peat based growing medium (PBGM)= Peat+ fertiliser+ground limestone – -General purpose mix 10 : 10 : 27 for example "Phostrogen" -Ground dolomite (magnesium) limestone, horticulture grade -Nutrient solution, prepare a solution containing approx. 50 mg/l nitrogen from the fertiliser by dissolving 0.5 g of the general purpose fertiliser in 1 l of water	-reference to this method - identification of the sample -total number emerged weed (?) -any observed abnormalities -average fresh weight per seedling for sample and PBGM -other details that affected the results
Federal Compost Quality Assurance Association, Germany (1998)	Determination and evaluation of plant tolerance (phytotoxicity) of compost by means of a germinating plant test with summer barley	See title	Summer barley	compost	Uniform soil 0 (EE0), also for mixing	Yields of fresh matter

Table 3: Summary of Experimental Design

Method	No. of Species	Seed treatments	Pot Size	No. of Seeds/Pot	Total No. of Plants/Pot	Total Number of Plants
ISO 11269-2	1 monocotyledonous and 1 dicocotyledonous species from a list of test plants (see above)	Not allowed	85-95 mm internal ∅	20	5 (after the emergence assessment: thinning of the seedlings to a total of 5 specismen)	20
ISO/CD 17126	1: Lettuce (*Latuca sativa L.*)	Seeds coated with insecticides and /or fungicides ("dressed" seeds) should be avoided	Plastic Petri dishes (∅15cm) or other containers with similar surface area (with fitting re-sealable polyethylene bags)	40		
OECD 208	3 (minimum) from a list of 16	Not specified	Non-porous plastic or glazed pots with a tray or a saucer under the pot (adequate for unrestricted growth)	Minimum of 5	Minimum of 5	20
CEN/TC 223	1: any of the following species: Lettuce (*Latuca sativa*), petunia *(Petunia hyb.)*, tomato (*Lycopersicum esculentum)*, geranium *(Pelargonium zonale)* or impatiens *(Impatiens hyb.)*	Seedlings are obtained in small cells, their content is the lump of transplanting	Horticultural pots with at least 10 cm ∅ and a minimum content of 350 ml; made of plastic, non porous and with a sufficiently openwork bottom to enable a good moistening; 1l pots (<20mm material)		1	
XP U 44-167 (French standardization – working document)	1 monocotyledonous and 1 dicocotyledonous species from a list of test plants (see above)	Not specified	Non porous plastic pots (cleaned and disinfected), inside ∅ 12-15 cm, volume 1 l	Depending from the species: 20 or 10	Depending from the species: 10 or 5	
ÖNORM 2021	1 cress *Lepidium sativum* (also - timothy – *phleum pratense*, -chinese Cabbage - *Brassica campestris L. var. chinensis*, - spring barley *(hordeum vulgare*	Not specified	Glass dishes ("Neubauerschalen") , ∅120mm, 60mm height	0.4g (±0.01g)		
VDLUFA, 1997 Evidence of phytotoxic substances	1 (Chinese cabbage or spring barley)	Seed dressing is possible, however, not necessary	Plastic dish ("Neubauer-Schale") ∅ 11-12 cm (100-110 cm^2), height 7-8 cm; Transparent plastic pots with holes, opaque cover pots with holes	Chinese cabbage: 30 Spring barley: 50		
VDLUFA, 1997 Evidence of gaseous phytotoxic substances	1, cress	Not specified	1 l glasses	Not specified	Not specified	Not specified
Federal Compost Quality Assurance Association, Germany (1998)	1, spring barley	Not specified	Plastic pots (500 ml) with bottom holes and trays	50 (counting or weighing: ±10mg after multiple weighing of 50 grains)		
WRAP, The Composting Association (2002)	2: **Lettuce**, Winter density and **Radish**, French breakfast **or Lentil** (as available from supermarkets	Not specified	-Plastic plant pots, 9cm (3.5inch) -Plastic plant saucers, 9 cm (3.5 inch) -Capillary matting, 3mm thick	3 pots with 8 seeds of lettuce and radish (or lentil) per pot		

Methods	No. of Replicates	Test Substance	Number of Doses	Increment between Doses	Treatment Method
ISO 11269-2	4	Do not use surfactants	Sufficient to obtain LOEC	geometric series factor not exceeding 2 *[1]	Mixed into soil
ISO/CD 17126	3	Contaminated soil or other contaminated samples (soil materials, compost, sludge)	Minimum 5	Geometric series, dilution factor not exceeding two. The range of concentrations should include those at which 0 (or minimum) and 100% emergence are expected, e.g. based on a preliminary test	lettuce seeds are pressed gently into the medium
OECD 208	4	Chemicals, crop protection products	3	10x; 0.1 to 1000 mg/kg dry soil	Mixed into soil
CEN/TC 223	3 (pots per elementary plot and 5 repetitions per treatment)	Growing medium			At the 2-3 leaves stage the transplanting of the seedlings in the test substrate is made
XP U 44-167 (French standardization – working document)	4	Organic soil improvers added to the test soil	3 increasing concentrations	D_1 (lowest quantity) is determined in relation to the standard quantity recommended by the manufacturer, $D_2 = D_1 * 2$ $D_{10} = D_1 * 10$	Added to the soil improver
ÖNORM 2021	3 minimum	Growing media, Compost; <10mm	Compost must be mixed (25% and 50vol.% compost)		Seeds on the substrate with underlying Silica sand ; covering with silica sand
VDLUFA, 1997 Evidence of phytotoxic substances	2-4 (Minimum, higher with increasing coarse material)	soils, products of bark, compost, growing media	Composts should be mixed (75+25 and 50+50)		Chinese cabbage: evenly spread at the surface; Spring barley: push slightly into the test substance, thin cover with sieved test substance, moistening
VDLUFA, 1997 Evidence of gaseous phytotoxic substances	3	Mineral soils (<2mm), peat soils (<5mm), other coarse substrates (<10mm)			1 g cress evenly spread on the surface of the substrate and also cress on a piece of moistened cotton wool
Federal Compost Quality Assurance Association, Germany (1998)	3	Compost < 10mm	Compost is mixed (25 and 50 %)		After seeding covering with 100 ml test mixture or reference material (controls)
WRAP, The Composting Association (2002)	3	Sample must be diluted with vermiculite to an EC of 400 ±50 µS cm^{-1}			Each seed: pressed with seed dibber below the surface until it is no longer visible

Table 4: Summary of the horticultural practices applied

Method	Duration	Soil characteristics	Microbially active	% OM/OC	Texture	pH	Watering	Fertilisation
ISO 11269-2	14-21 days. At least 2 weeks after50% of the seedlings have emerged in the control	Soil Sieved <4-5mm	Sterile or non-sterile	<3%/<1,5%	Particles passing through a 4-5mm sqare mesh; fine particles (<20 µm) shall not exceed 20% dry mass	5-7.5	Daily adjustment of the moisture conten to maintain a predetermined percentage water holding capacity (e.g. 80% for oat and 60% for rape); control by weighing	Yes, but not specified
ISO/CD 17126	• 7days	Not dried, sieved (<2mm or <5mm) with registered water content, water holding capacity, EC and pH					water is spread evenly over the surface to obtain appr. 85% of the water holding capacity	
OECD 208	At least 14-21days after 50% of the seedlings have emerged in the control	Soil shall be passed through a 5mm sieve	Yes	<3%/<1,5%	< 2mm; 10-20% clay	5-7.5	Bottom watering preferred, initial top watering can be used.	modified Hoagland nutrient solution or other appropriate nutrient source.
CEN/TC 223	4 weeks	Reference: A blend of perlite (20%) and white peat (80%)	Not specified	Not specified	fine granulo-metry (0-8mm);	pH: 5.5-7.5	Irrigation device, optimal condition for the chosen species (described in the annex); Subirrigation; Watertight support enables to bring water on a height of 2 cm and to drain the surplus (also plastic vat possible); additionally sprinkling possible	N, P_2O_5, K_2O *(1:0.5:1.4)*
XP U 44-167 (French standardization – working document)	14-21days after emergence and reducing the seedlings (to the half)	(standard) soil: sieved (<5mm),	Non sterile	C content: • 1,5%	Particles <0.02mm shall not exceed 20% of the dry mass	5-7.5	Soil moisture is regularly weighed. At the beginning the maximum water-retention capacity per pot is determined. During the test , when moisture reaches 70% of this maximum value, pot are watered by subirrigation.	Readily available nutrients should be high enough to ensure correctly nourishing of the plant species : N: 5mg/100g P: 1mg/100g K: 10 mg/100g (approximately) measured on aqueous extracts (1/5)

Method	Duration	Soil characteristics	Microbially active	% OM/OC	Texture	pH	Watering	Fertilisation
ÖNORM 2021	8-11 days after germination of the first plants in the reference	See test and reference material	Not specified			5-7	Moistening with gentle spraying or using a glass tube, no retained water	Low nutrient content
VDLUFA, 1997 Evidence of phytotoxic substances	14-21 days	See test and reference material; minerals soils sieved <2mm, peat and growing media< 5mm, compost<10mm	Not specified	1.5-2%	medium	6-6.5	Evenly moistening, casting with watering can, no water should leak or weighing (2g water each second day with 100 cm^2 surface)	Necessary analyses: pH, salinity, mineral N, Ca supply; Fertilisation of reference materials with low nutrient contents, possibly compost mixtures: NPK:14+16+18; 1g/l
VDLUFA, 1997 Evidence of gaseous phytotoxic substances	8 days	See test and reference material	Not specified	1.5-2%	medium	6-6.5	sensoric test via compression of the substances, slight moistening	Not specified
Federal Compost Quality Assurance Association, Germany (1998)	10-12 days	See test and reference material					Evenly moistening (about 60 ml) VE water; fist control	100 ml liquid NPK fertiliser (110 mg N/pot=220 mg N/l Substrat)
WRAP, The Composting Association (2002)	7 days for radishes 14 days for lettuce and lentil seedlings	See test and reference material	Not specified	-Vermiculite, horticultural grade -Sphagnum peat, medium grades; no higher than 2 on the von Post scale with a conductivity of < 5mS/m			Negative control is watered with the nutrient solution until fully wetted up, modified sample with deionised water. All subsequent watering to all the pots with deionised water. The surface of all pots shall be kept moist and the capillary matting shall also not become any wetter than moist. No water must pond above the capillary matting.	

Summary of the horticultural practices applied

Method	EC	Indoor/Outdoor	Lighting	Temperature	Relative Humidity	Covering
ISO 11269-2		Phytotron, plant growth room, greenhouse	Light intensity suitable for photosynthesis; Minimum 7000lux; 16 hours/day	temperature, humidity: for maintaining "normal" growth;		
ISO/CD 17126	EC of the sieved test material shall be determined	Controlled environment chamber	First 48 h in complete darkness, then 16h light, 8h dark , fluorescent light at 4300 lux ±430 lux.	Constant (±2°C) optimum temperature (for a given strain) for the germination of the lettuce seeds (20-24°C)		The contents of each Petri dish is covered evenly with 90 g dry cover sand. Between operations, the dishes are covered with lids in order to reduce evaporation. Immediately before the dishes are placed in polyethylene bags, the lid is removed.
OECD 208		Suitable facility	Suitable for maintaining normal growth	Suitable for maintaining normal growth	Suitable for maintaining normal growth	
CEN/TC 223	25-50mS.m^{-1} (extr. 1/5, v/v)	Greenhouse or growth room with appropriate climatic regulation fit to the optimal conditions of the chosen species (annex A)	optimal conditions of the chosen species (annex A)	optimal conditions of the chosen species (annex A)		
XP U 44-167 (French standardization – working document)	EC (ISO 11265) < 0.75 S/m	Phytotrons, controlled environment growth room and greenhouses	7000 lux/m^2 in wavelenghth suitable for plant growth, additional lightening may be required in greenhouse in winter(approx. 4000lux)	18-25°C	Should be recorded (greenhouse)	Not specified
ÖNORM 2021		Light room	Between October and March: additional source of light (e.g. 400 W Hg-vapour-lamp); 16 hours daylight	Room temperature		With glass plate and black plastic film
VDLUFA, 1997 Evidence of phytotoxic substances		Light room without direct insolation or green house or phytotron	Nov.-Feb.: additional lightening (12 hours with 3000 lx minimum)	16-20 °C		With glass lid, in the greenhouse: with paper or without, if chinese cabbage is the test plant and high humidity
VDLUFA, 1997 Evidence of gaseous phytotoxic substances	Examination of salt content (impairment of root growth at > 200mg salt/100g soil possible)	Light room or growth room without direct insolation or phytotron	Minimum 3000 lux ; 12 hours	18-22°C		With Glass lid
Federal Compost Quality Assurance Association, Germany (1998)		Air-conditioned room		18-20°C		covering with test or reference material (see treatment)

WRAP, The Composting Association (2002)	400 ±50 μS cm^{-1}	Greenhouse or plant growth chamber	10000 lux	15-25°C		All pots with opaque plastic film, e.g. black polythene sheeting

VALUATION OF EXISTING METHODS FOR DRAFTING A HORIZONTAL STANDARD

2.5 General approach

A multitude of current methods assesses the phytotoxicity of various materials on terrestrial, non target plants. Some methods – e.g. listed in reviews (Keddy, 1995, GCPF, 2000) - examine the phytotoxicity of either crop protection products (CPPs), general chemicals or both. These methods distinguish between germination tests and tests conducted on emerged plants. Germination studies are divided into two test design categories: seed germination/root (radical) length test and seed germination/emergence/growth test. In common these methods deal with the examination of the effect of added substances (CPPs or chemicals) on plants.

In this horizontal project the inherent phytotoxic effect of a certain material (soil, sludge, biowaste compost, additionally soil improvers and growing media and any kind of plant substrate) should be proved. Therefore only several aspects of these above mentioned methods, especially some details regarding the experimental design, will be considered for the evaluation of the methods and for the draft method proposal.

Generally, there are two possible approaches to assess phytotoxicity:

- to grow certain plants directly in the test material or in diluted samples
- to grow certain plants in hydroponic systems (e.g. rockwool granules) supplied with leachate or mixtures of leachate and nutrient solution

For the purpose of the draft method proposal the first approach to assess Phytotoxicity is used.

Furthermore, in some cases the use of closed systems is suggested to assess possible effects of volatile phytotoxins as well (e.g. one VDLUFA-method).

A difficulty lies in the fact, that the method shall be applicable on quite different materials and that test results will be used for different purposes. The optimal test design may probably differ according to the type of material to be tested and the purpose of the study.
In the tables 1 and 2 an overview of different plant test methods (principles, test plants, test and reference materials and test parameters) is given. A summary of the experimental design and the measures of Good Horticultural Practices is shown in the tables 3 and 4. In the following some comments concerning the key parameters of the different methods are given.

2.6 Test plants

The different methods use a multitude of potential test species. A more detailed list (18 monocotyledons and 11 dicotyledons) is included in the OECD-Guidelines (2000). Table 5 shows an assortment.

Depending on the scope of the method, several plants can be used for the test, ranging from monocotyledons like barley or ryegrass to dicotyledons like cress, lettuce or tomato. Differences exist in their sensitivity to different test materials and toxins as well as their applicability.

Also the number of test species suggested per guidance method varies (1 to 3).

2.7 Experimental design

There is a great variety of experimental designs available. The most important demands are

to offer sufficient rooting space (depending on the chosen crop and growth time)
to ensure the sufficient supply of water and
to ensure the sufficient supply of nutrients depending on the chosen crop and growth time

2.8 Test parameters

the obligatory key parameter of most methods is the

- fresh (and/or dry) weight of the shoot.

However, also

- the number of seedlings emerging
- the number of plants remaining at harvest
- the germination rate
- a valuation (development of cotyledons, colour, root health and –intensity, plant development)

as compared to the control are required.

Additionally, depending on the test plants several other criteria are suggested to allow a more detailed interpretation of possible phytotoxic effects:

Root weight
Root length
Development of the root system
Germination rate
Shoot/root ratio
Plant abnormities

In this draft, it is suggested to use some of these criteria in addition to the fresh (and/or dry) weight.

2.9 Reference material

All the parameters described above have to be related to the results obtained from a reference substrate. Again, a variety of materials is suggested by the different authors:

Sand
Rock wool granules
Perlite
Commercially available standardised substrates
Peat based growing media (PBGM) according to a specific composition
Commercially available substrates, low in nutrient content (e.g. for germination)
Mixtures of commercially available substrates with inert materials (e.g. tennis court sand)
Uniform soil (EE0)

However, the standard substrate has to be available any time all over Europe in constant quality.

2.10 Growing conditions

There is also a variety in growing conditions (like humidity, light, temperature) reported by the different authors (Table 4). The main task is to ensure optimal, comparable and constant germination and growing conditions for the plants.

In the following draft, the description according to ISO 11269-2 is proposed in the first place.

3. CRITICAL POINT AND RECOMMENDATIONS

3.1 Reference material

The reference material should both serve as a reference regarding plant growth and a possible mixing constituent for the test material as well. Therefore, an optimum range of water holding capacity and a sufficient nutrient content are necessary. It has been suggested in the comments, that the reference material should be without any plant nutrients, however a majority is of the opinion that it is very important for the reference being supplied with an optimal content of nutrients for the specific test species (e.g. according to VDLUFA Methodenbuch, 1997). If there is a reference material with low levels of nutrients compared with the test material (e.g. organic waste as compost or sludges), the potential adverse effects may be masked by their high levels of plant nutrients. However as the phytotoxicity test is only a qualitative approach, positive or negative effects cannot be assigned to certain parameters.

Mixtures of commercially available substrates with certain inert constituents like tennis court sand have proved to be suitable already. However, as the availability of these materials might be limited, research work has to be done to identify proper materials as mixing components or pure substrates available on a large scale (e.g. perlite). It has been suggested to specify a common reference material (e.g. also black peat:standard low nutrient substrate for sowing:quartz sand (0.3-0.8mm) = 5:5:1). Furthermore, weakly decomposed Sphagnum peat with defined properties has been proposed. As has been said before, the reference material has to be available locally and with uniform quality all over Europe.

Furthermore, possible ranges of top fresh (and/or dry) weight obtained by growing on the reference material have to be identified to enable the assessment of the performance of the test procedure. Below certain amounts of harvest, the results of the test must not be accepted. Also in this respect further research work will be necessary.

3.2 Fertilization

If initially a material is low in nutrients, deficiencies in plant growth might occur with no regard to phytotoxicity (see above). In this case, additional fertilization is thought to be necessary. To identify nutrient deficiencies, the determination of plant available N, P and K according to EN 13652 (water extraction) or EN 13651 (CAT-extraction) is suggested. For setting the thresholds (which should be species specific), additional research work is required. (Preliminary, this part is included in the draft but may be removed until the stating of final results of this research work.)
On the other hand the nutrients may interact with the pollutants and mask the toxic effects by improving the plant growth.

3.3 Salt Content, dilution of the test material

Some authors suggest a dilution of the test material in case of high salt contents/electric conductivity. As this parameter can be regarded as one of the most important factors of phytotoxicity (especially regarding sludges and composted biowaste), it has to be clarified whether there is phytotoxicity due to high salt content. Therefore the salt content expressed as electric conductivity has to be determined.

There are two different approaches:

1. The test material has to be diluted, if a certain value is exceeded.
2. Growth trials are only performed if EC is below a certain threshold.

In this field, further research work will be necessary.

To our opinion the material has to be tested undiluted in general. It has been stated, that dilution based on EC also leads to dilution of possible toxic effects. While testing a material only in diluted form, no assessment of the possible phytotoxic effect can be made as the maximum "dose" of the material of 100% is not reached. Besides, there might be additional phytotoxic parameters with no regard to the salt content. To enable a clarification in this respect, certain dilution ratios are proposed, always including the 100% variant.

With regard to the final use (e.g. compost in growing media, sludge) certain materials have be tested in dilution as well..

Therefore, the test design should meet the following requirements:

- Soils and growing media should be tested preferentially undiluted,
- Soil improvers and sludge should be tested after mixing with a soil at defined percentages,
- compost should be tested undiluted, diluted with soil or diluted with reference material (e.g. perlite).

3.4 pH

The pH contributes to the phytotoxicity of a material as well. However, sometimes a low or high pH might be required due to the purpose of the use. In these cases, if the initial pH of the material does not meet this demand, pH can be adjusted to the optimum range for the test plants (e.g. for growing media between 5 and 7).

3.5 Test plants

As pointed out before, there is a variety of test plants available to be chosen regarding certain methods or purposes. At least, the use of one monocotyledonous and one dicotyledonous species is proposed for the horizontal method to cover also possible effects of selective pesticides.

However the suggestions in the literature cover a wide range of possibilities:

- One test plant
  - Garden cress
  - Everything but garden cress
- Two or more test plants
  - Establishment of selection criteria (Justification for the use of monocotyledonous and one dicotyledonous)
  - No selection criteria, free choice

Without a prescribed choice of crops (e.g. Chinese cabbage and barley) no comparisons can be made. On the other hand a choice of one monocotyledonous and one dicotyledonous or a choice of 2-4 species to choose among, may be discussed as well. A higher number of species would lead to a lower practicability of the test. It was proposed that in regard to the various reasons for testing phytotoxicity special indicator test plants should be suggested for certain test purposes.

The choice may be up to the client or the performing laboratory, a request to the reason for using the selected species in the “Test report” (5.8.1.2) should be added.

A suggestion is included in the draft method.

3.6 Water holding capacity

Probably, the application of this method is limited to materials with sufficient water holding capacity. Materials with a large amount of coarse particles seem unlikely to provide an optimum moisture for growing seedlings. Again, there is an urgent need for further research work to define the scope of the method.

In the case of low water holding capacity 1+1 dilution with reference growth substrate enhances water holding capacity and could be suggested.

Furthermore, an exact definition of the optimum water content for plant growth is hardly possible. To ensure suitable moisture conditions, the water holding capacity test as described by VDLUFA (1997) by pressing the material through the fist can be proposed No water should be come out and the material must fit together. However, it is recommended that only staff experienced in plant growing should carry out the test.

4. DRAFT STANDARD (CEN TEMPLATE)

NOTE: Where italics appear in the draft method it indicates an area that requires additional work and confirmation

Due to the widespread use of the method and the differences in the tested materials there are many different and often contradicting approaches regarding the experimental design, the test plant, etc. In the following draft the most reasonable choices for certain parameters are listed and proposed for discussion.

4.1 Scope

This European Standard specifies a method to assess the phytotoxicity of soil, substrate, sludge and treated biowaste. The method is not applicable for materials with a water volume (EN 13041:1999) less than 50%.

4.2 Normative references

This method incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this method only when incorporated in it by amendment or revision. For undated references the latest edition of the publications referred to apply.

EN 00000 *HORIZONTAL Sampling procedures*

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 13041 Soil improvers and growing media - Determination of physical properties

ISO 11269-2 Soil quality – Determination of the effects of pollutants on soil flora – Part 2: Effects of chemicals on the emergency and growth of higher plants

4.3 Terms and definitions

to be discussed

4.4 Principle

The germination and development of indicator plants in the test sample is monitored in relation to a standard substrate under controlled growing conditions.

4.5 Reagents

4.5.1 washed quartz sand, particle size £ 3 mm.

4.5.2 Reference material: e.g.

- washed perlite, particle size 0.5-2 mm;

- mixture of equal masses of commercially available growing medium with low nutrient content (suitable for germination) and sand (with particle size • 2mm, particles < 0,063 mm less than 15%, max. limit for content of CaO or CaCO3: 5% of DM and a high degree of cleaning), e.g. tennis court sand

- EE0 (with a defined constitution of nutrients to get comparable results in different substrates).

The properties of reference materials must be well known and controlled.

4.6 Apparatus

4.6.1 Non porous PE- or glass trays, diameter = approximately 120mm, height = approximately 60mm (e.g. “Neubauer – tray”), for certain seeds (e.g. barley) greater pots are feasible.

It is under discussion, if porous plastic plant pots should be used with tray under the pot, where excess water drains out. The loss of possible phytotoxic substances is possible then. A filter paper or a fleece must be put on the bottom, if a perforated plastic pot is used.

4.6.2 glass or plastic tube, height app. 60 mm, inner diameter 6 – 8 mm (optional)

4.6.3 glass plate for covering the glass trays

4.6.4 opaque plastic film for covering the glass trays

4.6.5 balance, capable of weighing accurately to 0,01g

4.6.6 testing facility: phytotron, plant growth room or greenhouse

4.7 Sample preparation

The sampling is performed in accordance with EN 00000 (HORIZONTAL sampling procedure), the sample is prepared in accordance with EN 13040:1999, clause 8. Initially, the sample has to be tested without dilution. Depending on the scope of the test, further dilution with the standard material is possible.

NOTE: For dilution, the following ratios (test material + reference material) are proposed:

25, 50, 75 and 100%

- Soils and growing media should be tested preferentially undiluted,
- Soil improvers, sludges and composts will have to be diluted depending on their future use

4.8 Procedure

4.8.1 Experimental Design

4.8.1.1 Preparation of the trays

Fill the tray (4.6.1) with app. 200g (app. 100ml) washed quartz sand (4.5.1) and spread it evenly on the bottom of the tray (optional). Place a small glass tube (4.6.2) vertically in the middle of the tray (optional) and add as much test sample as necessary to leave a distance of appr. 10 mm to the upper edge of the tray after gentle compression. Dry test samples have to be moistened before filling. The same procedure is carried out with the standard substrate.

The use of quartz sand on the bottom is considered important to ensure a certain amount of drainage in this closed system (non-porous pots).

NOTE 1: If the purpose of the material requires a pH less than 5 (e.g. growing medium for Azalea-culture) and the initial material meets this condition, the pH has to be increased to pH 5 to 6.5 with lime before starting.

NOTE 2: If the nutrient status of the test sample is low, it has to be adjusted using suitable fertilizers (see Appendix A)

4.8.1.2 Test plants

At least, one monocotyledonous (Category 1) and one dictyledonous plant (Category 2) has to be used as test plants (see Table 1). The use of Barley (Hordeum vulgare L.) and Chinese Cabbage (Brassica campestris L. var. chinensis) is suggested.

Table 555 Test plants (in accordance with ISO 11269-2)

Category	Test plant	Scientific name
1	Barley (spring or winter)	Hordeum vulgare L.
1	Rye	Secale cerale L.
1	Ryegrass, perennial	Lolium perenne L.
1	Rice	Oryza sativa L.
1	Oat (common or winter)	Avena sativa L.
1	Wheat, soft	Triticum aestivum L.
1	Sorghum, common (or shattercane or durra, white or millet, great)	Sorghum bicolor (L.) Moench
1	Sweetcorn	Zea mays L.
2	Chinese cabbage	Brassica campestris L. var. chinensis
2	Cress, garden	Lepidium sativum L.
2	Mustard, white	Sinapis alba
2	Rape (or rape (summer) or rape (winter))	Brassica napus (L.) ssp. napus
2	radish, wild	Raphanus sativus L.
2	Turnip, wild	Brassica rapa ssp. rapa(DC.) Metzg.
2	Bird’s foot clover, Fenugreek	Trifolium ornithopodioides L.

2	Lettuce	Lactuca sativa L.
2	Tomato	Lycopersicon esculentum Miller
2	Bean	Phaseolus aureus Roxb.

4.8.1.3 Preparation of the seeds

Spread the seeds (N° or weight depends on the suggested species, precise instructions will be given) evenly on the surface, cover with test material and finally with a thin layer of appr. 50 g (appr. 25 ml) inert material (e.g. quartz sand or a horticultural grade of perlite).
Small seeds can be mixed with the fine particle sand (used in the reference material) to facilitate even sowing. The seeds may be surface sterilized using either 70% ethanol or sodium-hypochloride.
Afterwards, the substrate is moistened using the tube (optional) or gentle spraying. Back water has to be avoided. For each test plant, 4 replicates have to be prepared both for the test sample and the standard substrate.

4.8.1.4 Growing conditions

The temperature, humidity and light conditions shall be such that they are suitable for maintaining “normal” growth of all selected species.

NOTE: The following conditions and procedures are recommended:

a) Temperature: to meet the normal growing conditions of the species selected (normally between 15°C and 25°C)
b) Lighting: 12-16 hours per day; 3000lx minimum light intensity in the wavelength suitable for photosysnthesis. Therefore, in a greenhouse, additional lighting may be necessary during times of low natural light. The pots shall be shaded from direct sunlight, when needed.
c) Moisture: The moisture of the substrate has to be kept constant by daily gentle spraying. To monitor the variation of water content, randomly weigh several trays during culture. The variation of the moisture content must not exceed 10%. Furthermore, monitor the moisture distribution within the test sample by visual control. If necessary, add water through the glass tube.
With porous plant pots the watering may be done by sucking from a watered tray (after this the pots must be put on a sieve for dripping off). For some materials complete watering to full water capacity may lead to a surplus of humidity which inhibits aeration of the substrate and therefore proper germination. Homogenous watering with the fist test has been proved to by sufficient accurate and practicable.
Weighting of all pots in the beginning and watering the trays daily to standard weight with a balance is recommended because standard humidity of the test sample is essential for the relevance of the test.

4.8.1.5 Performing of the test

All trays are covered with a glass plate (4.6.3) and an opaque plastic film (4.6.4) - until germination starts. Also a plexiglass plate or a PE foil may be used instead.

After germination of the first plants in the reference material, the covers are removed from all the trays. The trays are kept 8 to 14 days (depending on the test plant) in the testing facility, then the plants are cut near the substrate surface and the mass of the fresh plant (and/or dry)

- accuracy 0,1g - is determined. *If dry matter is determined, plants have to be dried in an oven between 80 and 105°C until a constant mass is obtained.*

4.9 Calculation and Expression of results

The mean fresh (and/or) dry mass of the test plants germinated on the test sample is given by Equation (1):

$$\overline{M}_{sample} = \left(M1_{sample} + M2_{sample} + M3_{sample} + M4_{sample}\right) \div 4 \qquad (1)$$

where

$\overline{M}_{sample}$ is the mean fresh (dry) mass of the test plants germinated on the test sample and

$M1-4_{sample}$ are the masses of the test plants germinated on the test sample repetitions

The mean fresh (dry) mass of the test plants germinated on the standard substrate is given by Equation (2):

$$\overline{M}_{std} = \left(M1_{std} + M2_{std} + M3_{std} + M4_{std}\right) \div 4 \qquad (2)$$

where

$\overline{M}_{std}$ is the mean fresh (dry) mass of the test plants germinated on the reference material and

$M1-4_{std}$ are the masses of the test plants germinated on the reference material repetitions

If the deviation between the mean and the individual repetitions is more than 15%, the results are not valid.

The percentage of plant fresh (dry) mass germinated on the test sample in relation to the plant mass (fresh and dry) germinated on the reference material (PM), is given by equation (3)

$$PM\,(\%) = \frac{100 \cdot \overline{M}_{sample}}{\overline{M}_{std}} \qquad (3)$$

Furthermore, anomalies of plants or plant growth have to be recorded.

NOTE:

Additionally, it is recommended to monitor the following criteria as well:

a) germination rate in % compared to the reference material *(obligatory)*
b) delay of germination in days compared to the reference material *(obligatory)*
c) root growth: health of roots, percentage of fresh root weight of test sample in relation to reference sample.

4.10 Precision

No data available at the moment.

4.11 Validity of the test

The results are considered to be valid, if in the control pots 80% of healthy seedlings emerge. The valuation of the results is dependent on the purpose of the test.

4.12 Test Report

The test report shall include the following information:

A reference to this method
A complete identification of the sample (inclusive nutrient content)
The kind of diluent
The dilution ratios
The percentage of top fresh and dry mass germinated on the test sample in relation to the plant fresh mass germinated on the standard substrate.

The EC of the test material and the reference material as used in the pots.

The health of roots and possible the percentage of fresh root weight of test sample in relation to reference sample.

Germination rate
Identifications of the test plants and the reference material
Growth conditions
Table of data including separate replicates and the summary valuesAnomalies of plants or plant growth
Any deviations to the prescribed method.
Any factors that may have affected the test

It might be useful to provide an evaluation proposal, based on the fresh weight (and other criteria), which could be the basis for acceptance or non-acceptance of a material (alternatively a classification may be done). This needs further research.

NOTE: Further parameters as suggested in the NOTE in clause 4.9 may be included as well

APPENDIX A FERTILIZATION OF THE TEST SAMPLE

A.1 Introduction

Problems in germination and growth are not only a function of possible phytotoxic substances, but of nutrient supply as well. Whereas a nutrient surplus might be seen as a possible phytotoxic attribute, a lack of nutrients can be overcome easily by fertilizing. Therefore, prior to performing the test, the nutrient status of the test sample has to be investigated.

A.2 Test of the nutrient status

To assess the nutrient status of the test sample, the following determinations have to be carried out:

Determination of calcium chloride/DTPA(CAT) soluble nutrients in accordance with EN 13651

or

Determination of water soluble nutrients in accordance with EN 13652

Evaluation of the results

If the nutrient contents are below critical values, the sample has to be fertilized accordingly (Table 2).

Table 666

6 Threshold values for fertilizer requirement (species specific)

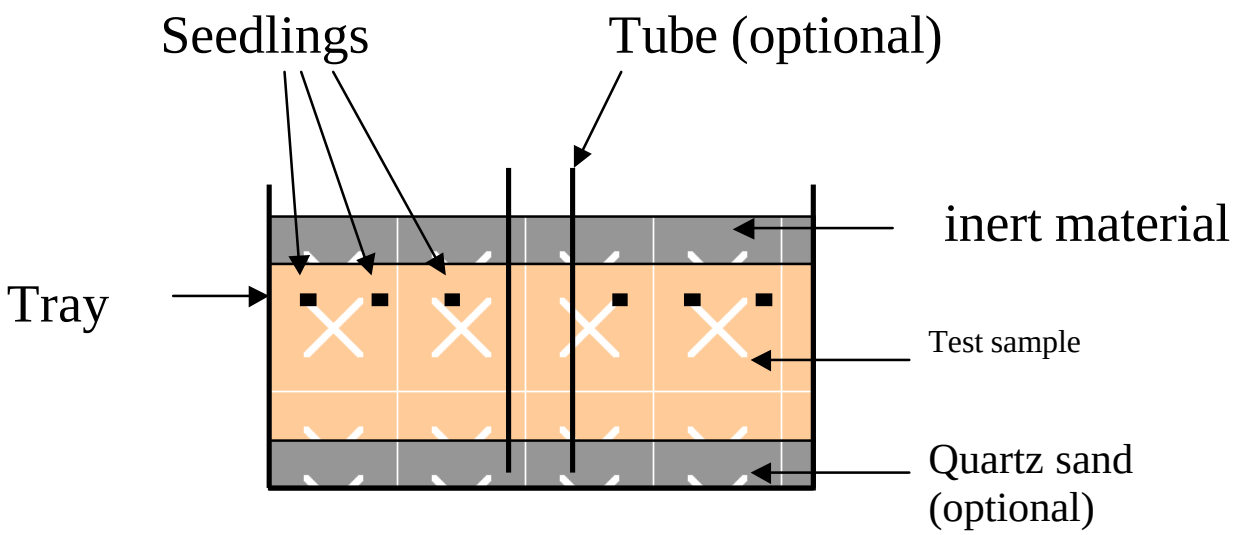
Nutrient	*lower limit CAT-extraction*	*lower limit Water-extraction*	*amount to be added (g/l)*	*possible fertilizers*
N				*NH_4NO_3*
P				*KH_2PO_4, H_3PO_4*
K				*K_2SO_4*

A.3 Test of EC (Cl^- and NH_3/NH_4)

Table 3 Max acceptable values for EC (Cl^- and NH_3/NH_4)

APPENDIX B EXPERIMENTAL DESIGN

Figure 1 Experimental design



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