
**Soil quality — Determination of
the toxic effects of pollutants on
germination and early growth of
higher plants**

*Qualité du sol — Détermination des effets toxiques des polluants sur
la germination et la croissance primaire des plantes supérieures*



COPYRIGHT PROTECTED DOCUMENT

© ISO 2016, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

	Page
Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	3
5 Reagents, test organisms and media	3
6 Apparatus and materials	4
7 Treatment and preparation of samples	6
8 Procedure	6
9 Measurement	8
10 Calculation of the percentage inhibition	9
11 Reference chemical	10
12 Precision	10
13 Validity criteria	11
14 Test report	11
Annex A (informative) Application of the phytotoxicity test in transparent test plates on natural and artificial soils and soil materials, with different plant species	12
Annex B (informative) Assemblage of test plates for the phytotoxicity test	14
Annex C (informative) International interlaboratory comparisons on the phytotoxicity test	15
Bibliography	20

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

Ecotoxicological testing of test soils or waste materials to be disposed on soil are required to assess the potential environmental risk resulting from soil pollution or the disposal of wastes such as sewage sludge on farmland. There is also a need to monitor the quality of soil after reclamation of industrial sites. Therefore, a very practical and rapid germination and growth test has been developed based on seed germination and seedling growth in controlled environmental conditions.

The assay, which does not require any pretreatment of the seeds, is performed in “transparent test plates”, incubated vertically, to allow the roots and the shoots of the germinated seeds to be seen. After 72 h exposure, a picture of the transparent test plates is taken and can be analysed “by image analysis” for multiple endpoints, such as percentage of seed germination and of length of roots and shoots. To account for the plant species variability in sensitivity, the assays are performed with the seeds of three plant species: one monocotyl (*Sorghum saccharatum*) and two dicotyls (*Lepidium sativum* and *Sinapis alba*).

A major advantage of this test is that after the shooting and storing of the pictures of the test plates, the measurements by image analysis can be postponed to any appropriate timing.

Reference or standard soils can be used as negative controls, such as, for example, the ISO standard artificial soil according to ISO 11269-1 and ISO 11269-2.

Commercially available seeds, with a shelf life longer than one year, allow the use of this test at any time of the year.

Two International interlaboratory comparisons demonstrated that the test provides good results.

A substantial number of studies report data on the application of this test on various types of soils and soil materials with several types of plant species.

Soil quality — Determination of the toxic effects of pollutants on germination and early growth of higher plants

1 Scope

This International Standard describes a technique for determining the effects of soil and soil-related materials on the seed germination and early growth of higher plants. These endpoints are useful indicators for the assessment of the quality of a soil as a habitat for organisms. This International Standard is applicable to all soils in which soil organisms are active and may be used to evaluate:

- the effects on plants due to toxicity of solid or liquid chemicals contaminating soil or materials (compost, sludge, waste) and chemicals added to soil;
- the changes in the soil effect on plants after restoration measures.

2 Normative references

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

ISO 11269-1, *Soil quality — Determination of the effects of pollutants on soil flora — Part 1: Method for the measurement of inhibition of root growth*

ISO 11269-2, *Soil quality — Determination of the effects of pollutants on soil flora — Part 2: Effects of contaminated soil on the emergence and early growth of higher plants*

ISO/TS 20281, *Water quality — Guidance on statistical interpretation of ecotoxicity data*

3 Terms and definitions

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

3.1

artificial soil

mixture of sand, kaolinite, peat and calcium carbonate prepared according to ISO 11269-1 and ISO 11269-2

3.2

control soil

reference or standard soil used as a control and as a medium for preparing dilution series with test soils or a reference substance

3.3

reference soil

uncontaminated site-specific soil (e.g. collected in the vicinity of a contaminated site) with similar properties (nutrient concentrations, pH, organic carbon content and texture) as the test soil

3.4

standard soil

field-collected soil or artificial soil whose main properties (e.g. pH, texture, organic matter content) are within a known range

Note 1 to entry: The properties of standard soils can differ from the test soil.