



SLOVENSKI STANDARD

oSIST prEN ISO 29200:2020

01-januar-2020

[Not translated]

Soil quality - Assessment of genotoxic effects on higher plants - Vicia faba micronucleus test (ISO 29200:2013)

Qualité du sol - Évaluation des effets génotoxiques sur les végétaux supérieurs - Essai des micronoyaux sur Vicia faba (ISO 29200:2013)

Ta slovenski standard je istoveten z: prEN ISO 29200

SIST EN ISO 29200:2020

<https://standards.iteh.ai/catalog/standards/sist/58cf47a8-35aa-4b0a-874e-f518a4e4a070/sist-en-iso-29200-2020>

ICS:

13.080.30

Biološke lastnosti tal

Biological properties of soils

oSIST prEN ISO 29200:2020

en,fr,de

INTERNATIONAL STANDARD

**ISO
29200**

First edition
2013-09-01

Soil quality — Assessment of genotoxic effects on higher plants — *Vicia faba* micronucleus test

*Qualité du sol — Évaluation des effets génotoxiques sur les végétaux
supérieurs — Essai des micronoyaux sur Vicia faba*

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Reference number
ISO 29200:2013(E)

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Published in Switzerland

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ISO 29200:2013(E)**Foreword**

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

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Introduction

In the field of assessment of the quality of soils and soil materials, it appears necessary to determine *in vivo* their genotoxic potential which may be induced by pollution or by a decontamination process. Indeed, genotoxic agents have the ability to damage the genome of living organisms or to interfere with its functioning, but they are not always detected by chemical analysis or classical ecotoxicological tests. Actually, genotoxic effects are often observed at sublethal concentrations, where no toxic effect (e.g. survival or growth) can be observed in the short term but some long term effects may be feared in living organisms. Moreover, higher plants, like *Vicia faba* (broad bean) are ecologically relevant to assess soils and soil materials quality.

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Soil quality — Assessment of genotoxic effects on higher plants — *Vicia faba* micronucleus test

1 Scope

The purpose of this International Standard is to describe a method for assessing genotoxic effects (chromosome breakage or dysfunction of the mitotic spindle) of soils or soil materials on the secondary roots of a higher plant: *Vicia faba* (broad bean). This method allows the assessment of genotoxicity (toxicity for genetic material) of soils and soil materials like compost, sludge, waste, fertilizing matters, etc. Two ways of exposure can be considered: a direct exposure of plants to the soil (or soil material) which is relevant for the real genotoxic potential and an exposure of plants to the water extract of the soil (or soil material). This last way of exposure to a leachate or an eluate allows the detection of the mutagens which are not adsorbed to soils and which may be transferred to aquatic compartments. Moreover, this test may be used to evaluate genotoxic effects of chemical substances and to waters, effluents, etc.

2 Normative reference

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 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis)*

ISO 11260, *Soil quality — Determination of effective cation exchange capacity and base saturation level using barium chloride solution*

ISO 11269-2:2012, *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 11465, *Soil quality — Determination of dry matter and water content on a mass basis — Gravimetric method*

ISO/TS 21268-1, *Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials — Part 1: Batch test using a liquid to solid ratio of 2 l/kg dry matter*

ISO/TS 21268-2, *Soil quality — Leaching procedures for subsequent chemical and ecotoxicological testing of soil and soil materials — Part 2: Batch test using a liquid to solid ratio of 10 l/kg dry matter*

EN 14735, *Characterization of waste — Preparation of waste samples for ecotoxicity tests*

3 Terms and definitions

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

3.1

control soil

uncontaminated substrate used as control and dilution medium for preparing dilution series with test soils or test materials

EXAMPLE compost, sludge, waste, chemicals

ISO 29200:2013(E)

3.2

mitotic index

number of cells in division per 1 000 cells observed when all of the stages of the mitosis are taken into account, from the prophase (when the chromosomes begin to condense) up to the telophase (when the chromatin of the two nuclei formed at each pole of the cell finishes decondensing).

3.3

test mixture

mixture of test material (soil, compost, sludge, waste or chemical) with control soil

4 Principle

This genotoxicity test is based on the detection of micronuclei in the cells of the secondary root tips of *Vicia faba* (broad bean). The micronuclei, visible in the cytoplasm of the cells, result from a chromosome break (effect of clastogenic substances) or from a dysfunction of the mitotic spindle (effect of aneugens).

In both cases, the fragments of chromosomes or the entire chromosomes cannot migrate to one of the poles of the spindle at the time of the anaphase of the mitotic division and therefore form one (or more) micronucleus.

The micronucleus frequency is determined in the control root cells and in those which have been exposed to the soil (or soil material) or the water extract of the soil being tested. A statistical test then enables to determine the significativeness of data.

5 Plants, test equipment and reagents

5.1 Equipment

The exposure of the plants to the soils and soil materials under test is performed in plastic pots (diameter: 9 cm, height: 10 cm).

Exposure to water extract of soils is carried out in glass containers (e.g. glass beaker of capacity 200 ml).

A microscope equipped with an objective with x 400 magnification is required for studying the microscopic effects of the cells.

5.2 Test organism

The plant selected for its high sensitivity to micropollutants and for its ease of obtention is *Vicia faba* (broad bean), Aguadulce, with a very long pod. This higher plant forms part of the family of pulses and of the dicotyledoneae class.

Seeds coated with insecticides and/or fungicides should not be used.

5.3 Reference substance

Maleic hydrazide is recommended as a reference substance. The positive control is carried out at the concentration of 10^{-5} M, 1,12 mg/kg and 1,12 mg/l for solid-phase and liquid-phase exposures respectively.

The preparation of this photodegradable substance as well as the exposure of the plant organisms to the solution shall be carried out in the dark.