INTERNATIONAL STANDARD

ISO 14238

First edition
1997-02-15

Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes

Qualité du sol — Méthodes biologiques — Détermination de la minéralisation de l'azote et de la nitrification dans les sols, et de l'influence des produits chimiques sur ces processus

Reference number
ISO 14238:1997(E)

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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 14238 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Annex A forms an integral part of this International Standard. Annex B is for information only.

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet: central@iso.ch
X.400: c=ch; a=400net; p=iso; o=isocs; s=central

Printed in Switzerland

Introduction

The soil consists of both living and non-living components which exist in a complex and heterogeneous environment. Microorganisms in the soil are mainly responsible for nutrient cycling and thus play an essential role in the maintenance of soil fertility. One of the most important microbial processes in soil is the mineralization of nitrogen in organic compounds to ammonium (ammonification) and thereafter to nitrite and nitrate (nitrification). Clearly, any long-term interference with this process could influence soil fertility.

This page intentionally left blank

Soil quality — Biological methods — Determination of nitrogen mineralization and nitrification in soils and the influence of chemicals on these processes

1 Scope

This International Standard specifies laboratory procedures for measuring the mineralization and nitrification of nitrogen by the soil microflora.

For investigations of a basic or advisory nature, outline procedures are given for evaluation of the rates and extent of N-mineralization in soil or soils of known or unknown quality.

For investigation of the potential toxicity of chemicals to N-mineralization in soils, a simple procedure is given which allows the impact of single chemicals to be assessed, and provides a basis for comparison of the toxicities of different chemicals.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on these International Standards are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10381-6:1993, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory.*

ISO 10390:1994, *Soil quality — Determination of pH.*

ISO 10694:1995, *Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis).*

ISO 11260:1994, *Soil quality — Determination of effective cation exchange capacity and base saturation using barium chloride solution.*

ISO 11261:1995, *Soil quality — Determination of total nitrogen — Modified Kjeldahl method.*

ISO 11274:—[1)], *Soil quality — Determination of the water retention characteristics — Laboratory methods.*

ISO 11277:—[1)], *Soil quality — Determination of particle size distribution in mineral soil material — Method by sieving and sedimentation following removal of soluble salts, organic matter and carbonates.*

1) To be published.

ISO 11465:1993, *Soil quality — Determination of dry matter and water content on mass basis — Gravimetric method.*

3 Definitions

For the purposes of this International Standard, the following definitions apply.

3.1 nitrogen mineralization (N-mineralization): Microbial degradation of an organic substance containing nitrogen, via the processes of ammonification and nitrification, to the respective inorganic end-products, specifically ammonium and nitrate.

3.2 ammonification: Microbial degradation of organic nitrogen to ammonia.

3.3 nitrification: Microbial oxidation of ammonium to nitrite and thereafter to nitrate.

3.4 inhibitory dose ($ID_{\%}$): Amount of a chemical added to soil that effectively inhibits N-mineralization by a stated percentage, after a given time, in comparison to an untreated control, for example, ID_{25} and ID_{50} indicate 25 % and 50 % inhibition of N-mineralization respectively.

4 Principle

The rates or extent of N-mineralization in aerobic soils are determined by measuring the concentrations of ammonium, nitrite and nitrate released during mineralization of nitrogen contained in the soil organic matter, or during mineralization of an added nitrogenous organic compound.

The influence of chemicals on N-mineralization is determined by amending soil with a readily degradable source of organic nitrogen, and measuring the percentage inhibition of product formation in subsamples treated with different quantities of a chemical as compared to an untreated control.

5 Materials

5.1 Soils

5.1.1 Selection of soils

5.1.1.1 Basic mineralization test

For basic tests used for comparing the N-mineralization capacities of different soils, or for comparing N-mineralization in one soil collected at different times of the year, ensure that the choice of soil(s) is consistent with the purpose of the determination.

5.1.1.2 Toxicity testing

For testing to determine the influence of chemicals on N-mineralization, use a soil with low content by mass of organic carbon (0,5 % to 1,5 %) and clay known to be capable of mineralizing added nitrogenous organic matter to the end-product (nitrate).

NOTE — Such soil represents a worst-case situation since absorption is minimum, and availability of the chemical to the microflora is maximum. For routine testing, soils with a pH less than 5 are not satisfactory as the rate of nitrification may be too low to permit a valid assessment of the effects of the chemical on the process.

5.1.2 Collection, handling and storage of soils

For all tests, the recommendations in ISO 10381-6 for collection, handling and storage of soil shall be followed.

The following information shall be documented:

— date of collection;

— date(s) used in experiments;

— storage conditions, including temperature, moisture content;

— length of storage.

5.1.3 Characterization of soils

To facilitate interpretation of data, and for comparative purposes, the following characteristics shall be determined.

a) Physical properties:

— particle size distribution measured in accordance with ISO 11277;

— water content in accordance with ISO 11465;

— water retention characteristic in accordance with ISO 11274 and/or water-holding capacity in accordance with annex A.

b) Chemical properties:

— pH of the soil in accordance with ISO 10390 or pH determined in KCl or $CaCl_2$ solution;

— cation exchange capacity (CEC) in accordance with ISO 11260;

— organic matter content in accordance with ISO 10694;

— total nitrogen content in accordance with ISO 11261.

5.2 Reagents and materials

5.2.1 Quartz sand, fine and clean, of particle size from 0,1 mm to 0,5 mm.

5.2.2 Potassium chloride, solution c(KCl) = 1 mol/l.

5.2.3 Nitrogeneous substrate, at a concentration which will give approximately 100 mg of nitrogen per kilogram of soil.

For example:

— lucerne meal, with a C:N ratio of the 16:1;

— horn meal;

— any other appropriate organic nitrogen source.

It is also allowable to measure mineralization of nitrogen from the soil organic matter. In this case, soil is not amended with an organic nitrogen source.

For tests in which nitrification alone is of interest, ammonium [$(NH_4)_2SO_4$] is an appropriate nitrogen source.

5.3 Test substance

A test substance is only needed where the purpose of the investigation is to determine if the substance can influence N-mineralization. Test substances shall be the purest that are commercially available. In many circumstances, it may be appropriate to test technical-grade or commercial-grade chemicals or mixtures.

NOTE — If carriers or formulation ingredients are mixed with the test substance, their influence on N-mineralization (if any) should be taken into account.

In standard experiments with known test substances, the following data (if applicable) shall be given:

— name (IUPAC);
— structure;
— Chemical Abstracts Service (CAS) number;
— relative molecular mass;
— purity;
— stability in water;
— solubility in organic solvents;
— vapour pressure;
— octanol/water partition coefficient (POW);
— acid dissociation constant (pKa);
— absorption coefficient (Koc).

6 Apparatus

Usual laboratory apparatus and the following:

6.1 Mechanical shaker.

6.2 Centrifuge or **folded filter paper:** (nitrate-free).

6.3 Instruments for measurement of concentrations of ammonium, nitrate and nitrite in soil extracts.

7 Procedures

7.1 Experimental options

7.1.1 Basic mineralization test

For comparing the N-mineralization capacities in different soils, or for comparison of N-mineralization in one soil collected at different times of the year, ensure that the design of the experiment and the analyses performed are consistent with the goals of the experiment.

7.1.2 Toxicity testing

To determine the influence of chemicals on N-mineralization, treat a single microbiologically active soil with at least five concentrations of the test substance. For convenience, limit the analyses to measurement of the quantities of nitrate formed (in milligrams of NO_3^- per kilogram of dry mass of soil) in treated and control samples after 28 days incubation. Using this simple test design, dose-response relationships can be established. In some cases, for example, where soil concentrations are known or can be predicted by rough screening (e.g. pesticides), dose-response information may not be needed and an untreated sample and one appropriate concentration of test chemical is sufficient.

7.2 Treatment of soils

7.2.1 Basic mineralization test

Choose a substrate from the list given in 5.2.3 although the final choice of specific organic substrate used depends on the purpose of the test. Mix the organic material chosen thoroughly and homogeneously into the soil. If

mineralization of nitrogen from the soil organic matter is being investigated, a nitrogeneous substrate need to be added.

7.2.2 Toxicity testing

To determine the influence of chemicals on N-mineralization, use any of the nitrogenous substrates given under 5.3.2.

NOTE — Compounds with low C:N ratios are probably the best choice as little of the nitrogen released during mineralization is immobilized by the soil microflora.

Mix the chosen nitrogenous substrate (5.2.3) thoroughly and homogeneously into the soil. Then, divide the soil into six subsamples of equal mass. Mix five of these subsamples with different concentrations of the substance to be tested (three replicates for each dose). Mix the remaining subsample, but do not add any test chemical (if a carrier is used, mix only into the soil). The chemical-free subsample serves as the untreated control. If possible, select a concentration series which allows ID_{25} or ID_{50} values to be estimated.

Apply the test substance using an appropriate carrier, for example:

a) in water, depending upon water solubility of the compound;

or

b) on a solid, for example mixed with quartz sand (5.2.1), or with a portion of the soil under investigation.

With many organic chemicals, the soil or sand used as a carrier can be coated with the test chemical by dissolving it in a solvent. In such cases, the solvent should be removed by evaporation before mixing with the soil.

7.3 Incubation of soils

For N-mineralization investigations, incubate the soils in either of these two ways:

a) bulk samples of each variant or treatment; or

b) as a series of individual subsamples of each variant or treatment.

When variants are incubated as bulk samples, prepare large quantities of soil and take subsamples (e.g. 10 g to 100 g) during the experiment, as needed. Here, the amounts of soil prepared are determined by the sizes of the samples taken, the number of replicates used and the duration of the experiment. Mix soils incubated in bulk thoroughly before subsampling. With large samples, spread the soil out to a depth of not more than 3 cm to facilitate oxygen transfer. Also mix the soil sample on a weekly bases.

When variants are held as a series of individual subsamples, divide each variant into a series of equal subsamples, and sacrifice these subsamples as needed. In studies with more than one sampling interval, prepare sufficient subsamples to account for all replicates and sampling times.

NOTE — The choice of temperatures, water content of the soil and light conditions during incubation will depend on the purposes of the experiment.

For tests to determine the influence of chemicals on N-mineralization, maintain soils at (20 ± 2) °C and a pore water pressure of approximately 0,02 MPa to the nearest 5 % [(40 ± 5) % to (60 ± 5) % of the maximum water holding capacity] in the dark.

NOTE — A temperature of (20 ± 2) °C has been chosen as a standard for comparative purposes and because it gives relatively rapid results. Temperatures outside this range can be used if they are more appropriate (for example, because of local conditions and lack of cooling equipment).

In all experiments, vessels holding soils shall allow free exchange of gases. This helps prevent the development of anaerobic sites which could cause nitrogen losses through denitrification. Minimize water losses from the soil by incubating soils in covered vessels. Determine the moisture content of the soil at regular intervals, and replace losses with deionized water.

NOTE — Deionized water can be applied to the surface of the samples as a fine spray.