SLOVENSKI STANDARD
SIST EN ISO 24032:2022

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Kakovost tal - Uporaba kletk s polži na terenu za oceno bioakumulacije onesnaževal (ISO 24032:2021)

Soil quality - In situ caging of snails to assess bioaccumulation of contaminants (ISO 24032:2021)

Bodenbeschaffenheit - In-situ-Käfighaltung von Schnecken zur Beurteilung der Bioakkumulation von chemischen Stoffen (ISO 24032:2021)

Qualité du sol - Encagement in situ d'escargots pour la mesure de la bioaccumulation de contaminants (ISO 24032:2021)

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Soil quality - In situ caging of snails to assess bioaccumulation of contaminants (ISO 24032:2021)

Qualité du sol - Encagement in situ d'escargots pour la mesure de la bioaccumulation de contaminants (ISO 24032:2021)

Bodenbeschaffenheit - In-situ-Käfighaltung von Schnecken zur Beurteilung der Bioakkumulation von chemischen Stoffen (ISO 24032:2021)

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CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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European foreword

This document (EN ISO 24032:2021) has been prepared by Technical Committee ISO/TC 190 "Soil quality" in collaboration with Technical Committee CEN/TC 444 “Environmental characterization of solid matrices” the secretariat of which is held by NEN.

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of an identical text or by endorsement, at the latest by June 2022, and conflicting national standards shall be withdrawn at the latest by June 2022.

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The text of ISO 24032:2021 has been approved by CEN as EN ISO 24032:2021 without any modification.

INTERNATIONAL STANDARD

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Soil quality — In situ caging of snails to assess bioaccumulation of contaminants

Qualité du sol — Encagement in situ d'escargots pour la mesure de la bioaccumulation de contaminants

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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).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological characterization,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 444, *Environmental characterization of solid matrices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Snails are ubiquitous soil macroinvertebrates living at the interface soil, plants and air. Those pulmonate gastropod molluscs are phytophagous and saprophagous (trophic level of primary consumers and detritivorous). They ingest vegetation and soil, and crawl on the ground where they lay their eggs. Therefore, snails integrate multiple sources and routes of contamination (see Annex A, Figure A.1). Snails participate in exchanges with soil and are preyed upon by various consumers (invertebrates: glow-worms, ground beetle larvae, or vertebrates: birds, small mammals such as shrews, hedgehogs and humans).

Among snail species, the recommended species is *Cantareus aspersus* O.F. Müller 1774[1)] (synonyms: *Helix aspersa aspersa*, *Cornu aspersum*) also known as common garden snail, brown garden snail, garden snail, land snail, nicked name in French "Petit-Gris" (see Annex A, Figure A.2). This species is a stylommatophoran pulmonate gastropod molluscs of the Helicidae family, widely distributed across the world[9],[28]. This palearctic species can be acclimated to regions with different types of climate: Mediterranean, oceanic temperate, midcontinental temperate and even tropical. *Cantareus aspersus* (Müller, 1774) is of European origin and has been introduced into all parts of the world. It is now on all continents except Antarctica. On the other hand, the species is recognized as an agriculturally harmful snail in some countries and must be treated carefully.

Juvenile snails are already covered in ISO 15952[1] that describes how to assess ex situ, i.e. in laboratory conditions, toxic effect of chemicals or contaminated matrix on the survival and growth of juvenile (1 g fw).

Currently there is no standardized in situ bioassay allowing the assessment in the field of the transfer of contaminants from the environment to organisms of the soil fauna. Indeed, despite ISO 19204[3] (relative to the TRIAD approach) which recommends the application of three combined lines of evidence (chemistry, ecotoxicology and ecology) and highlights the interest of bioindicators of effect and accumulation as additional tools for site-specific ecological risk assessment, few bioassays are available for this purpose. As described in ISO 19204:2017, Annex A, measurements of bioaccumulation in plants or soil organisms are thus useful to:

— assess the effective bioavailability of soil contaminants to soil organisms;

— approach the food chain transfer and the risk of secondary poisoning of consumers.

In some cases, bioaccumulation can result in toxic effects but this is not always the case (see ISO 17402[2]).

Since farming is possible (see ISO 15952:2018, Annex B), snails with a known biological past can be used on the field to analyse bioavailability of contaminants present in the habitats (soil, plants, air) by measuring their accumulation in individuals caged and exposed for a determined period of time.

C. aspersus can be used either in the field [10],[12],[13],[15],[19],[22],[23],[27],[29],[30] or in the laboratory [14],[18],[20],[21] to assess the fate and transfer (i.e. environmental bioavailability, ISO 17402) of chemicals in soils. This soil bioindicator has been applied on numerous field sites[2)] to evaluate habitat and retention function of soils. This bioassay allows determining the bioavailability of chemicals to snails thanks to the measurement of their concentration in their visceral mass (which contain mainly the digestive gland and some other organs as described in Reference [16]). The visceral mass is the main site of contaminant accumulation in snails.

This document describes how to expose snails in situ for 28 days and how to prepare them until chemical analysis are performed to assess bioaccumulation in their viscera. This bioassay evaluates the transfer of contaminants from the environment to land snails.

1) Available from: https://inpn.mnhn.fr/espece/cd_nom/199863/tab/taxo.

2) Available from: https://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur/english/worksheet.php.

This test is applicable in the field (e.g. contaminated sites, amended soils, soils after remediation, agricultural or other sites under concern and waste materials) by caging snails for 28 days on the studied site/soil/waste. Snails integrate chemicals of all terrestrial sources (soil, plant, air). After exposure, concentrations of chemicals are measured in the visceral mass of snails.

Optionally, the method can be used in the laboratory (ex situ) to evaluate bioaccumulation of chemicals of snails exposed only to soil (see Annex I).

The results of a ring test performed in situ by six laboratories to assess the method of exposure and by four laboratories from exposure until to chemical analysis are shown in Annex H.

Soil quality — In situ caging of snails to assess bioaccumulation of contaminants

1 Scope

This document describes a method to assess the bioaccumulation of chemicals in snails, i.e. concentrations of metal(loid)s (ME) or organic compounds [e.g. polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs)] accumulated in their tissues.

This document presents how to prepare snails for caging in situ for 28 days, the in situ test design and then how to collect and prepare the snails until conservation and further analysis. If a kinetic study of accumulation is necessary, sampling of snails at different time-points during exposure is possible as well [13],[19],[22].

This document excludes analytical methods. Preparation (extraction and mineralization) of the samples and quantification of chemicals are not in the scope of the present document.

The method is applicable for soils under different uses (agricultural, industrial, residential, forests, before and after remediation, on potentially contaminated sites, etc.) and waste materials [8],[10], preferably with vegetation and/or humus cover.

The method is applicable subject to certain limits of temperature (frost-free period, i.e. mainly from April to October in temperate region).

Optionally (see Annex I), the method can be used in the laboratory to evaluate the accumulation of contaminants [and optionally, the sum of excess of transfer (SET) index for ME, PAH, PCB] of snails exposed only to soil.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
caging
closed microcosm allowing exposure of snails by various routes and several sources

3.2
bioaccumulation
phenomenon by which a chemical present in the medium accumulates in a living organism

Note 1 to entry: This phenomenon is observed when the rate of absorption exceeds the rate of elimination of the contaminant.

3.3
inactive snail
snail without any activity, generally under dry conditions where they glue on the walls of the box in which they are placed (generally just due to a simple dried mucus ring)

3.4
aestivation
snails kept inactive, under dry conditions, at a temperature of 15 °C to 20 °C

3.5
plot
characteristic and representative sub-area of the site

Note 1 to entry: The geographical coordinates of each plot should be recorded.

3.6
site
field place (or geographical entity) under study and where the microcosms are placed to assess the bioavailability of contaminants to snails

Note 1 to entry: The site can present one or more plot(s) and land use, i.e. a field, a pasture, a forest, an industrial site, a discharge.

4 Principle

Snails are caged in microcosms at the study site for 28 days. Fifteen sub-adult [(5 ± 1) g of the body mass] garden snails shall be placed in each microcosm. From the end of their breeding to their placement on the soil, they can be stored inactive in dry wooden boxes (round wooden boxes, approximately 12 cm in diameter and 4 cm in height; see Figure 1 and Figure B.2). They are woken from aestivation by spraying them with water a few hours before they are placed in the microcosms. Here, they are exposed to soil as well as plants that have grown on-site and ambient air in order to be under natural exposure conditions (climate hazards).

After exposure, the collected snails are brought back to the laboratory and starved for 48 h. During the starvation, faeces are removed every 24 h. Snails are then frozen at –80 °C. After thawing, the soft body is removed from the shell; the visceral mass and the foot (see Annex B, Figure B.1) are separated and prepared for chemical analysis to determinate internal concentration of chemicals. Main steps are presented in Annex B.

5 Test organism and equipment

5.1 Biological material

Test organisms shall be sub-adult snails (to avoid mass change during the exposure duration and the consecutive dilution of the bioaccumulation per the mass gain during the growth or the transfers of compounds to the eggs during the reproductive stages). The recommended species is the land snail *Cantareus aspersus* (Müller, 1774) which shall be 7 weeks to 12 weeks old, having a mean fresh mass of (5 ± 1) g.

NOTE 1 Optionally, the shell diameter can be measured (mean ± SD of 25 mm ± 5 mm; min/max of 20 mm/30 mm).

The snails shall be selected from synchronous breeding in order to form a population as homogeneous as possible with respect to mass and age. The breeding techniques for snails are described in Annex C. In summary, after a nursery and a growth period (3 weeks to 6 weeks followed by 4 weeks to 6 weeks), the sub-adult snails shall be used directly or after an aestivation period that should not be more than 5 months [i.e. snail inactive, fixed on the wall of a dry box (plastic box shall be avoided), in a temperature-controlled room between 15 °C and 20 °C]. The aestivation is carried out in round wooden boxes

(approximately of 12 cm in diameter and 4 cm in height; usually 15 snails per boxes, which is equal the number of snails per microcosm).

Snails shall be reared for the purpose of the project (see Annexes C and D) or be purchased from local snail farmers.

NOTE 2 The use of some other genus and/or species of *Helicidae* is possible (see examples and conditions in ISO 15952:2018, Annex G).

A control of the chemical quality of the subadult snails selected for the caging (i.e. unexposed snails) can be performed on 6 snails with respect to the initial concentrations of the chemicals of interest (C snail-t0). These control snails can be selected at the same time as the snails used for snail caging. The analysis of the chemical quality of snails before caging can be done at the same time as the analysis of snails after exposure. It is not mandatory to make this control. Indeed, after exposure, all data are compared to the threshold guide value (TGV) (see 8.2.1); however, if possible to get these data, it provides an indication that snails were uncontaminated before exposure. For chemicals for which no TGV are available, data can be compared to various values (see 8.2.2.4) among which are Csnail-t0.

The sub-adult snails used shall present usual concentrations in the visceral mass before caging (see Annex E). For PAH and PCB data, as extraction are often made on fresh tissues, the data of Table E.1 are in $\mu g.kg^{-1}$ fresh mass of viscera (these values can be converted in $\mu g.kg^{-1}$ dw on the basis of ≈ 15 % dry mass of the visceral mass); for metal(loids), the data are in $mg.kg^{-1}$ dry mass of visceral mass.

5.2 Equipment

5.2.1 Microcosm, stainless steel cylinders with 25 cm diameter and 25 cm height covered by a 0,5 cm or 1 cm mesh netting.

An example is presented in Figure 1 and in Annex F, Figure F.1.

NOTE 1 Other devices can be used if the material that constitutes them cannot be a source of contamination; for some purpose (e.g. exposure of snails to chemicals sprayed in the field), fully screened microcosm can be used [see for example Reference [11] that used stainless steel cages of 25 cm × 25 cm × 15 cm (mesh size of grid: 1 cm) closed by a stainless steel grid of 30 cm × 30 cm (mesh size: 1 cm) held by four pickets (see Annex F, Figure F.2)].

NOTE 2 In some cases, it can be necessary to protect the microcosm from predators or cattle (see examples in Annex F, Figure F.3) or from the sun (see Annex F, Figure F.4).

5.2.2 Netting, 0,5 cm or 1 cm mesh netting, also stainless steel.

5.2.3 Pickets, stainless steel picket (diameter 5 mm; length 46 cm to 72 cm) to maintain the mesh netting on the cage. Depending on the soil settlement or the presence of stones, the size of picket shall be adapted.

5.2.4 Pieces of tiles, see Figure 1 and Annex F.

5.2.5 Wooden storage. Inactive snails can be stored and transported before exposure in round wooden boxes (approximately 12 cm in diameter and 4 cm in height), with the snails under dry conditions, at a temperature of 15 °C to 20 °C (see Figure 1, Figure B.2 and Annex G).

5.2.6 Boxes for fasting, sampling. For the preparation of snails in the laboratory [e.g. to keep the snails before individual weighing), plastic containers (PCs) (e.g. made of transparent polystyrene or any other container having approximate dimensions: 24 cm (length) × 10,5 cm (width) × 8 cm (height)] can be used.

5.2.7 Calliper rule. For the measurement of the shell diameter, a calliper rule having a precision of 0,1 mm.