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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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Contents

Page

Foreword

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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.