SLOVENSKI STANDARD
SIST EN 17136:2019

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Kakovost vode - Navodilo za terenske in laboratorijske postopke kvantitativne analize in identifikacije velikih nevretenčarjev v celinskih površinskih vodah

Water quality - Guidance on field and laboratory procedures for quantitative analysis and identification of macroinvertebrates from inland surface waters

Wasserbeschaffenheit - Anleitung zu Feld- und Laborverfahren zur quantitativen Analyse und Identifizierung von Makroinvertebraten aus Inland-Oberflächengewässern

Qualité de l'eau - Guide sur les modes opératoires de terrain et de laboratoire pour l'analyse quantitative et d'identification des macro-invertébrés des eaux de surface intérieures

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Water quality - Guidance on field and laboratory procedures for quantitative analysis and identification of macroinvertebrates from inland surface waters

Qualité de l'eau - Guide sur les procédures de terrain et de laboratoire pour l'analyse quantitative et l'identification des macro-invertébrés des eaux de surface continentales

Wasserbeschaffenheit - Anleitung zu Feld- und Laborverfahren zur quantitativen Analyse und Bestimmung von Makroinvertebraten aus Binnenoberflächengewässern

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (EN 17136:2019) has been prepared by Technical Committee CEN/TC 230 “Water analysis”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by August 2019, and conflicting national standards shall be withdrawn at the latest by August 2019.

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Introduction

WARNING — Working in or around water is inherently dangerous. Care should be taken when working with different and generally toxic preservatives. This standard does not purport to address the safety problems associated with its use. It is the responsibility of the user to adopt appropriate health and safety practices in compliance with national regulatory conditions.

Macroinvertebrates are important in water quality assessment and form one of the biological quality elements in the EC Water Framework Directive (WFD 2000/60/EC). Macroinvertebrates are a very diverse group of organisms for which an efficient and effective procedure for analysis is not always obvious. Their large size range, very different appearances and the diverse range of habitats they live in produce specific problems for collecting and analysis. This standard aims to provide a general procedure for the quantitative analysis of macroinvertebrate samples for the WFD in particular and scientific studies in general. It is not intended to give a full and exact overview of all possible methods. Although specific situations and investigation objectives may require adjustments to the outlined procedure the general principles given in this guideline should still be valid.

1 Scope

This document gives guidance on the quantitative estimation of abundance and identification of macroinvertebrates in samples taken from inland waters. The procedure deals with pre-treatment (cleaning), sub-sampling, sorting, and final identification of organisms from preserved and unpreserved samples originating from natural habitats or artificial substrates and their transport to the laboratory. Specific guidance is given for preservation for DNA-analysis.

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:

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

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

3.1
fixation
protection from disintegration of the morphological structure of organisms

3.2
macroinvertebrate
invertebrate that is easily visible without magnification (> 0,5 mm) and generally belongs to the group of organisms that live for at least one life stage on or in the bottom substrate or vegetation of inland surface waters

3.3
matrix
components of a sample other than the macroinvertebrates

3.4
preservation
process that protects organic substances from decay

4 Principle

The identification and quantification of macroinvertebrates comprises both field and laboratory procedures. After an optional pre-treatment of rinsing and sieving in the field, macroinvertebrate samples are transported to the laboratory where the samples are further processed. Macroinvertebrates are taken out of the matrix material by a sample dependent technique, quantified and sorted in functional groups for identification using a microscope or DNA-analysis. Several techniques exist to clean (remove unwanted matrix material) the sample and sort the organisms. The most suitable technique should be selected by visual inspection of the individual sample. Dependent on the objective of the analysis samples can be preserved in the field or cooled and processed unpreserved.

5 Equipment, preservatives and reagents

5.1 Sieves

Each new sieve should be checked for the right mesh size before first use. The smallest (typically 500 µm) sieve (having a significant effect on the validity of the analysis) is critical, its mesh should be guaranteed by the manufacturer attestation or control, providing metrological traceability. It should regularly visually be inspected for visible damage before every use

For DNA-based studies the removal of larger, visible contaminants by washing the equipment in water only is not sufficient. Instead sieves should be cleaned preferably with an appropriate bleach solution for each consecutive sample location.

5.2 Microscope

A low-power stereo-zoom microscope (typical magnification ~10x to 60x) can be used for accurate quantitative sorting and most identifications. For the species identification of specific groups like Chironomidae and Oligochaeta a compound microscope (typical magnification 40x to 400x) should be used.

5.3 Fixatives and preservatives

5.3.1 General

There is a distinct difference between fixation and preservation. In general, fixation is preferred for morphological analysis. Within the context of freshwater macroinvertebrate analysis ethanol as a preservative is the most often used chemical for preserving samples for quantification and identification. When ethanol is used as a preservative formaldehyde fixation can be left out for macroinvertebrate analysis.

WARNING — Formaldehyde is hazardous to humans (CMR substance, see ECHA) even at very low concentrations in air. Formaldehyde fixation should be avoided as much as possible.

Preservation by ethanol and formaldehyde fixation affects the estimation of biomass. Although weight loss by ethanol is considered to be more significant than by formaldehyde no unambiguous scientific data are available on the exact effect of either preservative. Therefore, prior to estimating biomass on the basis of preserved organisms the bias by using preserved material should be assessed for the specific taxonomic groups under investigation and the relevant conditions of sampling and preservation.

After the fixative or preservative has been added the sample should be gently turned to disperse the preservative.

5.3.2 Formaldehyde, 37 % (volume fraction)

Formaldehyde 37 % should be added to an undrained sample with a final concentration of 4 % to 6 %. This concentration provides a good fixation of organisms (avoiding damaging and shrinking). Formaldehyde is not a suitable fixative for DNA analysis. Formaldehyde samples should always be thoroughly rinsed with water on a sieve prior to processing.

Long-term storage in formaldehyde can seriously affect the colour of specimens and dissolves the shells of Mollusca (if not buffered). To prevent this, formaldehyde fixed samples can be transferred to 70 % ethanol. Transferring the sample to ethanol can already be done after 24 h of fixation. The sample should be thoroughly rinsed with water to remove the free formaldehyde before adding the ethanol.

5.3.3 Ethanol, C_2H_5OH

Before addition of ethanol the sample should be drained to minimize the dilution with excess sample water. In most cases ethanol 96 % should be added to compensate for dilution by the residual sample water. In samples with absorbing material and/or relatively large organisms (e.g. numerous molluscs) the initial 96 % ethanol should be replaced after a few days to ensure a permanent concentration of approximately 70 % in the sample. The sample container should not be filled for more than one third with material. Ethanol should be added until the sample is completely immersed and the container is filled to achieve a concentration of 75 %. Ethanol concentrations well above 70 % will harden many organisms rendering them difficult to manipulate and identify. Ethanol concentrations below 70 % will lead to tissue degradation.

Initial ethanol concentrations should be checked sample-wise with an alcohol meter.

Unfixed macroinvertebrate samples preserved with ethanol can be stored for several years.

Macroinvertebrate samples used for DNA studies or DNA-based identification that need to be stored for extended times before analysis (e.g. DNA barcoding) should be placed within two days of sampling in ≥ 90 % ethanol and preferably stored in a cool and dark place. At concentrations below 80 % DNA will degenerate.

5.4 Reagents for examination using compound microscopes

5.4.1 General

Many chemicals are known to enhance the microscope image quality in order to be able to accurately describe diagnostic features. The most relevant chemicals including mounting media can be found in group specific identification literature. Koenike and Laevulose syrup, presented below, are the most often used reagents. Koenike can also be used as a preservative for Arachnida (mites).

5.4.2 Koenike

Mix 50 ml of glycerol with 30 ml of water and add 20 ml of acetic acid. This clear solution can be stored at room temperature for an unlimited period of time.

5.4.3 Laevulose syrup

Dilute 25 g D(-)Fructose in 25 ml water by proper stirring (magnetic stirring bar). The solution can be slightly heated. Thereafter add 25 ml of lactic acid and stir again. This clear solution can be stored at room temperature for an unlimited period of time.

6 Pre-laboratory procedure

6.1 General

The pre-laboratory procedure starts with a visual inspection of the sample to assess the amount of matrix material (silt, clay, sand, macrophytes) and the number of organisms. With this information the right procedure for pre-treatment and sorting can be selected. It can be done in the field directly after sampling or in the laboratory.

The general objective is to select the organisms in the most effective way (least amount of time). If sub-sampling is necessary it should be done in a random way. When not all organisms have to be identified selectivity in sorting of taxa should be avoided.

Before collecting the organisms the sample should be rinsed and cleaned and/or sub-divided in smaller portions.

In case of unpreserved samples macroinvertebrates are collected alive in larger trays with the naked eye or with the use of a magnifying glass.

In case of preserved samples the macroinvertebrates with the remaining matrix are transferred to small trays and collected with the use of a stereo-zoom microscope.

Each collected organism is first sorted to its main taxonomic group (see Table 1). In a second step, the organisms are identified per taxonomic group to the desired taxonomic level.

Generally the smallest mesh size for collecting the macroinvertebrates during the analysis is 500 µm (e.g. sampling EN ISO 10870) as for most waters it is the best combination for trapping the macroinvertebrates and at the same time allowing effective rinsing. In specific situations (e.g. clear stony rivers) a smaller mesh size for maximum trapping of small macroinvertebrates can be selected.

For samples intended for DNA analysis, special emphasis should be put on working with clean equipment during the whole process to minimize the risk of cross-contamination during both field and laboratory procedures.

If macroinvertebrates are sorted alive, the sorting should preferably be done within 24 h (max 48 h) and measures should be taken to prevent predation [1] and dying of organisms by oxygen depletion during transport and storage. By this and by logistic considerations preservation as soon as possible after sampling is often preferred. In case of preserved samples it can be advantageous to select specific groups (e.g. Platyhelminthes) from the live sample in the field for identification to anticipate the negative effects of nonspecific preservation for these groups.

6.2 Sample processing in the field

The fresh sample should first be put on a large sieve (typically 500 µm mesh size) to rinse with water or if clean enough (as is often the case with handnet samples) directly in a white tray. It can also be directly transferred to a bucket if it is decided to process the sample completely in the laboratory (see 6.3).

Typical initial field activities are:

— removing of large wood, plant particles and stones after being accurately inspected for clinging or sessile organisms;

— preservation of specific taxa in appropriate preservatives;

— sorting and identification of vulnerable, large, easy recognizable and rare taxa which can be released again in the field;

As organisms (not only small) can actively pass through a 500 µm sieve, speed in processing is required.

After pre-treatment the sample should be immediately cooled and/or preserved. As alternative to cooling and preservation samples can also be frozen at ≤ −18 °C. However, freezing is not suitable for some taxa with fragile integuments (for example Platyhelminthes).

6.3 Transport and storing of samples

Samples should be stored in watertight plastic sample bottles and/or buckets. During transport unpreserved samples should contain only a minimum amount of adherent water and should be cooled to temperatures of (3 ± 2) °C in a cool box or in a refrigerated car to prevent rapid deterioration by oxygen depletion.

Limiting the amount of adherent water is an effective measure in preventing predation by immobilizing predatory organisms [1] during transport and storage. Nevertheless, predation stays a critical aspect of transporting and storing unpreserved samples.

If no vapour tight containers are used ethanol preserved samples can also better be transported and stored at temperatures of (3 ± 2) °C to prevent evaporation of the ethanol.

7 Laboratory procedure

7.1 General

Prior to sorting pre-treatment of the sample might be necessary to effectively reduce the amount of matrix material and/or the number of organisms that have to be identified per group. The procedure (Figure 1) should start with expert judgement of the sample to decide in what way the sample has to be processed if the sample has not yet been visually inspected in the field. Several combinations of the described methods are possible to optimize the analysis insofar selectivity and non-random sorting is prevented.