



# SLOVENSKI STANDARD

## SIST EN 16414:2014

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### Kakovost zraka - Biomonitoring z mahovi - Pasivni monitoring akumulacije zračnih onesnaževal z mahovi: od zbiranja do priprave vzorcev

Ambient air - Biomonitoring with mosses - Accumulation of atmospheric contaminants in mosses collected in situ: from the collection to the preparation of samples

Außenluft - Biomonitoring mit Moosen - Akkumulation von Luftschadstoffen in Moosen (passives Monitoring): Probenahme und Probenaufbereitung

Air ambiant - Biosurveillance à l'aide de mousses - Accumulation des contaminants atmosphériques dans les mousses prélevées in situ: de la récolte à la préparation des échantillonss

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EUROPEAN STANDARD

**EN 16414**

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## Ambient air - Biomonitoring with mosses - Accumulation of atmospheric contaminants in mosses collected in situ: from the collection to the preparation of samples

Air ambiant - Biosurveillance à l'aide de mousses -  
Accumulation des contaminants atmosphériques dans les  
mousses prélevées in situ: de la récolte à la préparation  
des échantillons

Außenluft - Biomonitoring mit Moosen - Akkumulation von  
Luftschadstoffen in Moosen (passives Monitoring):  
Probenahme und Probenaufbereitung

This European Standard was approved by CEN on 13 December 2013.

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
COMITÉ EUROPÉEN DE NORMALISATION  
EUROPÄISCHES KOMITEE FÜR NORMUNG

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## Contents

Page

Foreword.....	3
0 Introduction .....	4
0.1 Biomonitoring and air quality.....	4
0.2 Biomonitoring and EU legislation .....	4
0.3 Biomonitoring with <i>in situ</i> mosses .....	5
1 Scope .....	6
2 Terms and definitions .....	6
3 Principle of the method.....	7
4 Equipment .....	7
4.1 Field equipment .....	7
4.2 Laboratory equipment.....	8
5 Sampling design .....	8
5.1 General.....	8
5.2 Monitoring regional patterns of deposition .....	8
5.3 Monitoring localized emission source .....	9
6 Sampling strategy.....	9
6.1 General.....	9
6.2 Moss species.....	10
6.3 Period of collection.....	10
6.4 Sampling unit characteristics.....	10
7 Sampling procedure .....	11
7.1 General.....	11
7.2 Moss sample .....	11
7.3 Sample collection .....	11
7.4 Packing .....	11
8 Sample preparation .....	12
8.1 Sample cleaning.....	12
8.2 Sample homogenization .....	12
8.3 Sample storage .....	12
9 Recommendations for sample analysis .....	12
10 Quality Control and Quality Assurance.....	12
10.1 General.....	12
10.2 Quality Control .....	13
10.2.1 Overall variability .....	13
10.2.2 Interspecies calibration.....	13
10.2.3 Storage of the samples .....	13
10.3 Quality Assurance .....	13
Annex A (informative) Example of a survey sheet.....	14
Annex B (informative) Example of the location of the sampling units near an emission source.....	15
Annex C (informative) List of main moss species used in published bioaccumulation studies .....	16
Bibliography .....	17

## Foreword

This document (EN 16414:2014) has been prepared by Technical Committee CEN/TC 264 "Air quality", 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 2014, and conflicting national standards shall be withdrawn at the latest by August 2014.

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**EN 16414:2014 (E)****0 Introduction****0.1 Biomonitoring and air quality**

The impact of air pollution is of growing importance worldwide. Local and regional assessment is necessary as a first step to collect fundamental information, which can be used to avoid, prevent and minimize harmful effects on human health and the environment as a whole. Biomonitoring may serve as a tool for such a purpose. As the effects on indicator organisms are a time-integrated result of complex influences combining both air quality and local climatic conditions, this holistic biological approach is considered particularly close to human and environmental health end points and thus is relevant to air quality management.

It is important to emphasize that biomonitoring data are completely different from those obtained through physico-chemical measurements (ambient concentrations and deposition) and computer modelling (emissions data). Biomonitoring provides evidence of the effects that airborne pollutants have on organisms. As such it reveals biologically relevant, field-based, time- and space-integrated indications of environmental health as a whole. Legislation states that there should be no harmful environmental effects from air pollution. This requirement can only be met by investigating the effects at the biological level. The application of biomonitoring in air quality and environmental management requires rigorous standards and a recognized regime so that it can be evaluated in the same way as physico-chemical measurements and modelling in pollution management.

Biomonitoring is the traditional way through which environmental changes have been detected historically. Various standard works on biomonitoring provide an overview of the state of the science at the time, e.g. [1], [2], [3] The first investigations of passive biomonitoring are documented in the middle of the 19th century: by monitoring the development of epiphytic lichens it was discovered that the lichens were damaged during the polluted period in winter and recovered and showed strong growth in summer [4]. These observations identified lichens as important bioindicators. Later investigations also dealt with bioaccumulators. An active biomonitoring procedure with bush beans was first initiated in 1899 [5].

**0.2 Biomonitoring and EU legislation**

Biomonitoring methods in terrestrial environments respond to a variety of requirements and objectives of EU environmental policy primarily in the fields of air quality (Directive 2008/50/EC on ambient air, [6]), integrated pollution prevention and control (Directive 2008/1/EC, [7], and Directive 2010/75/EU, [8]) and conservation (Habitats Directive). The topics food chain ([9]) and animal feed ([10], [11], [12]) are alluded to as well.

For air quality in Europe, the legislator requires adequate monitoring of air quality, including pollution deposition as well as avoidance, prevention or reduction of harmful effects. Biomonitoring methods appertain to the scope of short and long-term air quality assessment.

Directive 2004/107/EC of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air ([13]) states that “the use of bio indicators may be considered where regional patterns of the impact on ecosystems are to be assessed”.

Concerning IPPC from industrial installations, the permit procedure includes two particular environmental conditions for setting adequate emission limit values. The asserted concepts of “effects” and “sensitivity of the local environment” open up a broad field for biomonitoring methods, in relation to the general impact on air quality and the deposition of operational-specific pollutants. The basic properties of biomonitoring methods can be used advantageously for various applications such as reference inventories prior to the start of a new installation, the mapping of the potential pollution reception areas and (long-term) monitoring of the impact caused by industrial activity. The environmental inspection of installations demands the examination of the full range of environmental effects. For the public authority, biomonitoring data contribute to the decision-making process, e.g. concerning the question of tolerance of impacts at the local scale.

The Habitats Directive (92/43/EEC on the conservation of natural habitats and of wild fauna and flora [14]) requires competent authorities to consider or review planning permission and other activities affecting a European designated site where the integrity of the site could be adversely affected. The Directive also

provides for the control of potentially damaging operations, whereby consent may only be granted once it has been shown through appropriate assessment that the proposed operation will not adversely affect the integrity of the site. The responsibility lies with the applicant to demonstrate that there is no adverse effect on such a conservation area. For this purpose, biomonitoring is well suited as a non-intrusive form of environmental assessment.

As an important element within its integrated environmental policy, in 2003 the European Commission adopted a European Environment and Health Strategy ([15]) with the overall aim of reducing diseases caused by environmental factors in Europe. In Chapter 5 of this document it is stated that the “community approach entails the collection and linking of data on environmental pollutants in all the different environmental compartments (including the cycle of pollutants) and in the whole ecosystem (bio-indicators) to health data (epidemiological, toxicological, morbidity)”. The European Environment and Health Action Plan 2004-2010 ([16]) which followed the adoption of this strategy focusses on human biomonitoring, but emphasizes the need to “develop integrated monitoring of the environment, including food, to allow the determination of relevant human exposure”.

### 0.3 Biomonitoring with *in situ* mosses

Mosses in the strict sense are non-vascular plants belonging to the *Bryophyta* phylum. They are composed of a leafy stem (or gametophyte) bearing reproductive organs and one or more sporophytes, made up of a capsule attached to the end of a stalk that grows out of the gametophyte. According to the morphology of the moss and the position of the sporophytes, mosses are sorted into the pleurocarpous or acrocarpous main types.

For most mosses, the lack of roots, vascular system, or protective cuticle means that water and nutrients come mainly from dry, wet and occult deposition. Therefore contaminant levels in tissues of terrestrial mosses originate mainly from the atmosphere. The high surface-to-volume ratio, the large contact surface due to many leaves overlapping around the stem, as well as thin leaves (made of a single cell-layer), enable mosses to trap particles efficiently. As a result, particulate and dissolved air contaminants are taken up and retained by mosses, either on leaf surfaces or inside moss tissues. For these reasons, terrestrial mosses have been commonly used in air monitoring programmes as bioaccumulators of a wide range of atmospheric contaminants, particularly mineral compounds and elements, especially metals but also organic substances (persistent organic pollutants) and radioactive isotopes ([17], [18]).

**EN 16414:2014 (E)****1 Scope**

This European Standard describes the sampling protocol and the preparation of samples of *in situ* mosses to monitor the bioaccumulation of atmospheric contaminants.

This European Standard specifies the actions that shall be taken from the field sampling of mosses to their final preparation before analysis for targeted contaminants.

This European Standard is of interest to all operators wishing to conduct air quality biomonitoring studies.

**2 Terms and definitions**

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

**2.1  
biomonitoring**  
use of biological systems (organisms and organism communities) to monitor environmental change over space and/or time

Note 1 to entry: Biological systems can be further considered as bioindicators.

**2.2  
bioindicator**  
organism or a part of it or an organism community (biocoenosis) which documents environmental impacts

Note 1 to entry: It encompasses bioaccumulators and response indicators.

**2.3  
bioaccumulator**  
organism which can indicate environmental conditions and their modification by accumulating substances present in the environment (air, water or soil) at the surface and/or internally

**2.4  
response indicator**  
effect indicator  
organism which can indicate environmental conditions and their modification by either showing specific symptoms (molecular, biochemical, cellular, physiological, anatomical or morphological) or by its presence/absence in the ecosystem

**2.5  
acrocarpous moss**  
moss with gametophyte producing sporophyte at apex of a stem or main branch, which generally grows erect in tufts (rather than mats) and are sparsely or not branched

[SOURCE: Bibliographical reference [19], modified — The definition has been grammatically changed so that it can replace the term in context.]

**2.6  
pleurocarpous moss**  
moss producing sporophytes laterally from a perichaetial bud or a short specialized branch rather than at the stem tip

Note 1 to entry: With stems usually prostrate, creeping and freely branched moss growing in mats rather than tufts.

[SOURCE: Bibliographical reference [19]]



**2.7*****in situ* moss**

moss naturally present in the study area

**2.8****terrestrial moss species**

terricolous mosses growing on soil, corticolous and epiphytic mosses on tree bark and branches and saxicolous mosses on rocks and walls, excluding aquatic and semi-aquatic species (e.g. *Sphagnaceae*)

**2.9****background level**

concentration of a substance in samples exposed and/or collected in a part of the study area, where no emission source has a local influence

Note 1 to entry: To help characterize the background deposition, either measured or modelled data can be used.

**2.10****sampling unit**

circular or square area that is ecologically homogeneous and no smaller than 100 m<sup>2</sup>, determined for the sampling of mosses

**2.11****subsample**

smaller portion of the target moss population to compose the sample related to the sampling unit

**2.12****study area**

geographical area considered by the study; it should be described in detail in terms of extent, land use classification and altitudinal range

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**2.13****stratified random sampling design**

technique consisting of subdividing a heterogeneous population into sub-populations (strata), which are more homogeneous and mutually exclusive

Note 1 to entry: Within each stratum the samples are consequently independent and randomly selected.

**3 Principle of the method**

This European Standard describes a method of sampling *in situ* mosses for measuring concentration of elements, mineral and organic compounds in mosses.

This European Standard applies to spatial and/or temporal biomonitoring of atmospheric contaminants. It allows spatial and temporal comparisons to be made. It can be used to identify and localize one or more pollution sources and to monitor the background pollution levels. This European Standard applies to local or large scale studies.

**4 Equipment****4.1 Field equipment****4.1.1 Positioning equipment.**

The position of the sampling unit should be identified using any tool that allows the highest obtainable accuracy. It should provide geographical coordinates by a degree / minute / second system or any correlating coordination system. These data can be provided by GPS, maps with high resolution (1:25 000 or 1:10 000 or 1:5 000 if available), and/or aerial photographs with coordination systems.

**EN 16414:2014 (E)****4.1.2 Moss determination equipment.****4.1.2.1 Magnifying glass (x 10).****4.1.2.2 Identification keys.****4.1.3 Sampling equipment.**

Non-talc gloves; sampling materials and containers inert towards the chemical contaminants that are to be assayed; icebox for the storage of moss samples for measuring concentrations of organic compounds, assay and survey sheet (example in Annex A).

**4.2 Laboratory equipment****4.2.1 Standard laboratory equipment.**

Tweezers, binoculars, microscope, microscope slides and cover slips.

**4.2.2 Determination keys.****4.2.3 Laboratory equipment necessary for moss preparation.**

Bench, heater, grinding material if necessary, freezer if necessary, balance.

**5 Sampling design**

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**5.1 General**

Selection of the most suitable sampling design is based on consideration of a balance between the accuracy required and the associated time and cost restrictions. There is no unique sampling design, notably concerning the number and location of sampling units and the extent of the study area. Decisions shall be based on study area specific information and objectives of the study.

Some preliminary steps shall be taken before selecting the most appropriate sampling design:

- the distribution of potentially suitable moss species over the study area shall be predetermined as far as possible, by means of a preliminary inspection throughout the study area;
- in case of gradient studies (e.g. prevailing point source of pollution or wind direction and intensity), quantitative information concerning the gradient shall be considered and, if possible, a thematic layer map should be produced;
- any kind of restricted access (e.g. private estates and military areas) shall be preliminarily checked in order to include or exclude those areas from the study;
- check by means of topographic maps and aerial photos if so far disregarded further emission sources (traffic routes, agricultural plants, industrial plants, quarries, dumps, etc.) that could affect the investigation result are located in the environment of the sampling area.

Different sampling designs can be proposed according to the scope of the biomonitoring project: monitoring of regional patterns of deposition or monitoring of the impact of a localized emission source (e.g. waste incinerator, power station, industrial plant).

**5.2 Monitoring regional patterns of deposition**

In large scale monitoring studies where no assumptions can be made about contaminant deposition, it is recommended that either systematic or random sampling design be used ([20]). The number and distribution

of sampling units vary according to the dimensions and characteristics of the study area, the study objectives, the financial support ([21]) and the desired level of interpretation of the results (statistical analysis and mapping method).

In ecologically heterogeneous areas, a stratified random sampling design is recommended to describe the whole study area adequately. For this, the different strata are determined from the information available on the heterogeneity of the ecological parameters (e.g. altitudinal maps, land use classification). Subsequently, sampling units shall be randomly allocated within each stratum in proportion to its dimensions.

To carry out temporal monitoring, the same sampling design shall be maintained. It is recommended to collect the moss samples from the same sampling units that were used in previous studies. If this is not the case, appropriate solutions should be considered, depending on the sampling scheme adopted (e.g. in the case of systematic sampling, the sampling unit can be moved by a fixed distance from the previous one; in the case of random sampling another sampling unit can be selected).

### 5.3 Monitoring localized emission source

The sampling design shall reflect the variability introduced by field characteristics at the landscape level (e.g. topography, land cover, location of other emission sources) and should take into account sites for which data on emission sources or meteorological parameters (e.g. precipitation, wind direction and velocity) are already available. The sampling designs commonly used are:

- concentric rings around the emission source point to be monitored; the sampling units are located at the intersections between the circle and its radius;
- transect from the emission source to be studied; the main directions shall take into account the highest probability of contaminant fallout (dominant wind direction, topography, altitude).

To assess the impact that an emission source can have on its vicinity, the sampling design shall contain:

- Location of sampling units at increasing distances from the emission source to observe a potential gradient of deposition; for example, these sites can be located on a straight line running from the emission source along the most frequent wind direction.
- Location of sampling units giving information about the presence of additional pollution sources in the vicinity of the monitored source; for example, sampling units can be located towards the least frequent direction of the wind.
- Location of sampling units reflecting the regional background levels of contamination and not influenced by the emission source: sites shall be located far from all known sources of isolated pollution. To be eligible, these sites shall be located in an area showing similar climatic and environmental conditions to those in the vicinity of the emission source and be investigated during the same year.

Although the number of sampling units depend on individual studies (increasing the number of sampling units provides greater reliability of results), it is recommended that an adequate number of sampling units be used (a specific example is given in Annex B).

To carry out temporal monitoring, see 5.2.

## 6 Sampling strategy

### 6.1 General

It is important to follow guidelines concerning the moss species to be collected, period of sampling and sampling unit characteristics in order to control and reduce the effects of environmental parameters other than air pollution on the contaminant concentration in mosses.