SLOVENSKI STANDARD
SIST EN 16413:2014

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Kakovost zraka - Biomonitoring z lišaji - Kartiranje raznovrstnosti epifitskih lišajev

Air quality - Biomonitoring with lichens - Assessing epiphytic lichen diversity

Luftqualität - Biomonitoring mit Flechten - Kartierung der Diversität epiphytischer Flechten

Qualité de l'air - Biosurveillance à l'aide de lichens - Evaluation de la diversité de lichens épiphytes

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13.040.20 Kakovost okoljskega zraka Ambient atmospheres

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Ambient air - Biomonitoring with lichens - Assessing epiphytic lichen diversity

Air ambiant - Biosurveillance à l'aide de lichens - Evaluation de la diversité de lichens épiphytes

Außenluft - Biomonitoring mit Flechten - Kartierung der Diversität epiphytischer Flechten

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Foreword

This document (EN 16413:2014) 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of an identical text or by endorsement, at the latest by August 2014, and conflicting national standards shall be withdrawn at the latest by August 2014.

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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 be met only 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 lichens

Many lichens, due to their morphological, ecological and physiological peculiarities, are extremely sensitive to changes in their environment ([17], [18]) such as eutrophication ([19], [20]), climate ([21], [22]) and woodland management ([23], [24]).

Lichen diversity is an excellent indicator of pollution from phytotoxic gaseous substances ([18], [25]). Lichens respond relatively fast to a deterioration in air quality and can re-colonize urban and industrial environments as a consequence of changing conditions within a few years, as recorded in many parts of Europe (e.g. [26], [27]).

The method described here determines the actual state of lichen diversity before or after exposure to air pollution and/or other types of environmental stresses. Correlative studies between lichen diversity and epidemiological studies suggest that bioindicators can be useful tools for detecting the possible effects of air pollution on human health ([28]).

This European Standard proposes a standardized method to assess lichen diversity on tree bark and is largely based on the German VDI standard on lichen mapping ([29], [30]), the French national standard ([31]), the Italian guidelines ([32], [33]) and the publication by *Asta* et al. ([34]). The interpretation of geographic patterns and temporal trends of lichen diversity may be assisted by using ecological indicator values ([35], [36], [37], [38]), multivariate statistics, such as numerical analysis of matrices of species ([39], [40]), non-parametric models ([41], [42]) or other statistical tools.

1 Scope

This European Standard aims to provide a reliable, repeatable and objective method for assessing epiphytic lichen diversity. According to international literature on the topic (see e.g. [18] for an overall outline), it provides a framework for assessing the impact of anthropogenic intervention, particularly for estimating the effects of atmospheric pollution.

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 considerd 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
lichen
ecologically obligate, self-supporting symbiotic association of a fungus (the mycobiont, generally an ascomycete) and one or more populations of green algae and/or cyanobacteria (the photobionts), which results in a stable vegetative structure (“thallus”) with a definite morphology

2.6
lichen community
biocoenosis
assemblage of populations of lichens, whose composition and aspect is determined by the properties of the environment and by their relationship with other epiphytes, animals, etc.

2.7
lichen diversity
species richness found on the bark of standard trees at a height ranging between 1 m and 1,5 m, above the base of the trunk at four different aspects (NSEW)

Note 1 to entry: See Annex B.

2.8
epiphyte
plant or plant-like organism growing on another plant, dependant on mechanical support but not deriving nutrients from the plant upon which it grows

2.9
study area
geographical area considered by the study

Note 1 to entry: It should be described in detail in terms of extent, land use classification and altitudinal range.

2.10
study domain
geographical extent in which the target population is studied

Note 1 to entry: It may coincide with the study area or it may be more restricted.

2.11
sampling point
geographic location identified by a pair of geographic coordinates (Lat, Long), being the reference point for the Sampling Unit, selected on the basis of a given sampling design

2.12
sampling unit
SU
either single tree or cluster of trees (tree-based sampling) or plot (geographical area of determined size, centred on a sampling point) where data are collected

2.13
probabilistic sampling
sampling conducted according to the statistical principles of sampling

Note 1 to entry: The essential principle of probabilistic sampling is that every individual particle or item in the population has an equal chance of being sampled.

2.14
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.

2.15
target population
lichen communities living on the bark of standard trees at a height ranging between 1 m and 1,5 m, starting from the base of the trunk at each main aspect

Note 1 to entry: Standard trees should be defined in terms of species, bole circumference and inclination (see below) and should be located within the study area.

3 Principles

The procedure is widely applicable for the purposes of collecting lichen diversity data. The interpretation of the results, however, shall be adapted to the regional characteristics of the lichen flora and to the prevalent types of environmental stress. Different methods may be used to solve particular problems, or in particular areas.

For quality assurance purposes, investigations performed according to this European Standard require personnel or institutions to have the necessary level of expertise in the fields of lichenology and probabilistic sampling design.

4 Equipment

4.1 Field work preparation equipment

4.1.1 Maps.

The choice of the map scale depends on the study area dimension and on the intended use of the map. Different scale maps will be necessary, both small scale maps for the study areas (e.g. 1:250 000 scale map) and large scale maps (at least 1:25 000 scale maps but also 1:10 000 and 1:5 000 scale maps) that may be useful for the location of the SU in the field.

4.1.2 Geographic Information System (GIS) with land use strata based on the Corine Land Cover nomenclature.

Other important sources of information may be the analysis of aerial photographs, of town and country planning maps, or ecological maps. The topography may also be used as an additional stratum in those regions presenting significant variation in topographical relief.

4.1.3 Identification of SU on the 1:25 000 (or more detailed if necessary) scale map.

The limits of the SU, and of their possible replacements, will be drawn on the map in order to facilitate field work.

4.1.4 Algorithm for random sampling (scientific calculator or statistics software).

4.2 Field equipment

4.2.1 Chemical reagents for spot tests on lichen thalli.

To produce characteristic colour changes. In particular, the most commonly used reagents are calcium hypochlorite (C), potassium hydroxide (K) and para-phenylendiamine dilution (P).

4.2.2 Compass-clinometer, essential to find the correct positioning of the observation grids on the trunk of the selected trees and also to measure bole inclination.

4.2.3 Identification keys.

Keys may be useful to distinguish species in the field (see Bibliography).

4.2.4 Envelopes.

Specimens to be transported to the lab for identification should be placed in separate, labelled envelopes. The use of paper envelopes is recommended to avoid the growth of mould on the lichen samples.

4.2.5 Global Positioning System (GPS) receiver.

4.2.6 Knife.

This is important to remove lichen samples from the bark of selected trees.

4.2.7 Magnifying glass.

It is essential to have a lens that magnifies by at least × 10 but a × 20 lens is also recommended for crustose lichens.

4.2.8 Maps.

See 4.1.1.

4.2.9 Tape measure: at least a 20 m tape measure: useful for measuring tree circumference.

4.2.10 Observation grid, 10 cm × 50 cm grid (Figure 1), subdivided into five 10 cm × 10 cm quadrats, to be applied to the trunk of sample trees for example by means of rubber bands.

The grid shall be flexible enough to be easily placed on the bole but also sufficiently robust and resistant so as to prevent changes in shape and in dimensions with use.

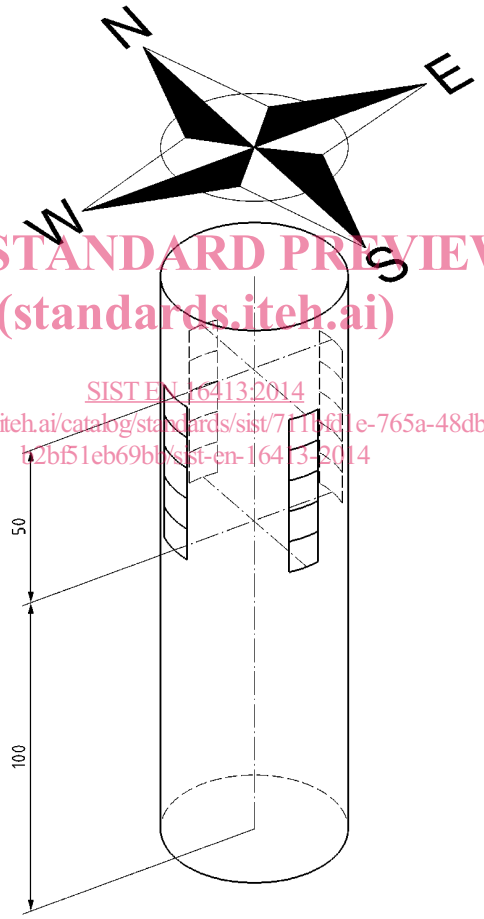

Figure 1 — Observation grids attached at four aspects along the trunk (see 5.4.8)

4.2.11 Survey sheets: Sampling Unit survey sheet (see Annex A).

4.3 Laboratory equipment

4.3.1 Chemical reagents traditionally used in lichenology.

Solutions of iodine (I), calcium hypochlorite (C), and potassium hydroxide (K), para-phenylendiamine (P). For solution preparation procedures refer to *Purvis* et al. ([43]). Blotting paper to highlight the spot test reaction on

lichen thalli. For the determination of several critical genera, such as *Lecanora*, *Pertusaria*, *Parmotrema*, *Cladonia*, *Lepraria*, some thin layer chromatography (TLC) analysis may be necessary and should be carried out according to the method suggested by *Culberson* ([44]) and *Culberson* et al. ([45]).

4.3.2 Identification keys (see Bibliography).

4.3.3 Online lichen checklists (see Bibliography).

They may be useful for nomenclature and ecological and distributional information of lichen species found in the field.

4.3.4 Optical microscope (required magnification: × 400 up to × 1 000), used for high-power magnification of lichen structures such as asci and spores.

An eyepiece micrometer will also be necessary to measure spore dimensions. Polarisator appliance is also recommended for the determination of several groups of species (e.g. *Lecanora* spp.).

4.3.5 Stereo microscope (minimum range × 10 to × 60), used for low-power magnification of lichen samples.

4.3.6 Usual small laboratory equipment (tweezers, scalpel or razor blades, microscope slides and cover slips, immersion oil, pipettes).

5 Sampling

5.1 General

Sampling is the act or process of selecting a part (the sample) of something (the target population), with the intent of reflecting its quality, style and nature. Since there are many possible designs, and the most effective one depends on the nature of the population being investigated, there is no unique sampling design that can be recommended for all studies. Rather, the probabilistic nature of the sampling design shall always be maintained. The following guidelines are provided in order to drive the main steps in defining the sampling design for individual studies. A synthetic flow chart showing the main steps to be followed is provided in Annex F.

5.2 Sampling objective

The sampling objective is to obtain an estimate of the parameter of the response variable (e.g. mean species richness or mean Lichen Diversity Value; LDV) over the study domain with a given precision. The precision level should be expressed in terms of confidence intervals for the defined probability level. It is required that the sampling objective is defined for each study.

EXAMPLE Obtain an estimate of the mean LDV for the study domain with a confidence interval ± 10 % of the mean value, at a probability (P) level of 95 %.

The computation of estimates and confidence intervals depends on the sampling design adopted. Therefore each study shall define precision and probability levels, taking into account the requirements of the study framework and considering the available resources.

5.3 Study type considered

Lichen diversity assessment and monitoring is a typical observational, mensurative study. Studies can be classified with respect to their temporal coverage:

a) the study is a baseline;