SLOVENSKI STANDARD
SIST EN 16789:2017

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Zunanji zrak - Biomonitoring z višjimi rastlinami - Metoda standardizirane izpostavljenosti tobaka

Ambient air - Biomonitoring with Higher Plants - Method of the standardised tobacco exposure

Außenluft - Biomonitoring mit Höheren Pflanzen - Verfahren der standardisierten Tabak-Exposition

Air ambiant - Biosurveillance à l'aide de Plantes Majeures - Méthode de l'exposition de tabac standardisée

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Ambient air - Biomonitoring with Higher Plants - Method of the standardized tobacco exposure

Air ambiant - Biosurveillance à l'aide de plantes supérieures - Méthode de l'exposition normalisée du tabac

Außenluft - Biomonitoring mit Höheren Pflanzen - Verfahren der standardisierten Tabak-Exposition

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Contents

European foreword

This document (EN 16789:2016) 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of an identical text or by endorsement, at the latest by February 2017, and conflicting national standards shall be withdrawn at the latest by February 2017.

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Introduction

0.1 General

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

It is important to emphasize that biomonitoring data differ from those obtained through physico-chemical measurements (ambient concentrations and deposition) and computer modelling (emissions and dispersion 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 as robustly as physico-chemical measurements and modelling in pollution management.

Biomonitoring is the way through which environmental changes have historically been detected. 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 address a variety of requirements and objectives within EU environmental policy, primarily in the fields of air quality (Directive 2008/50/EC on ambient air quality and cleaner air for Europe [6]), integrated pollution prevention and control (Directive 2010/75/EU on industrial emissions IED [7]) and conservation (Habitats Directive). It is also relevant to the topics food chain [8] and animal feed [9; 10; 11].

For air quality in Europe, legislators require adequate monitoring of air quality, including pollution deposition as well as avoidance, prevention or reduction of harmful effects. Biomonitoring methods are relevant for both short-term 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 [12] states that "the use of bio indicators may be considered where regional patterns of the impact on ecosystems are to be assessed".

With respect to IED for 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 opportunities for application of biomonitoring methods in relation to the general impact on air quality and the deposition of installation-specific pollutants. The basic properties of biomonitoring methods can be used advantageously for applications such as reference inventories prior to the start of a new installation, mapping of the potential pollution reception areas and (long-term) monitoring of the impact caused by industrial activity. The environmental inspection of installations demands examination against a range of environmental effects. For the competent 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 [13]) requires competent authorities to assess or adapt planning permission and other activities affecting a site designated at the European level 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.

In 2003, as an important element within its integrated environmental policy, the European Commission adopted a European Environment and Health Strategy [14] with the overall aim of reducing diseases caused by environmental factors in Europe. Chapter 5 of this document states 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 (bioindicators) to health data (epidemiological, toxicological, morbidity)". The European Environment and Health Action Plan 2004-2010 [15] which followed the adoption of this strategy focuses 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 Development of the standardized tobacco exposure

Ozone is a phytotoxic gas, which is a secondary pollutant formed in the atmosphere. It can lead to growth losses in plants and therefore to reduced yields in agriculture [16; 17; 18; 19; 20; 21; 22; 23]. Ground-level ozone also contributes to the development of forest decline [24; 25; 26; 27]. Effects of ozone on wild plants are the subject of numerous investigations [e.g. 28; 29; 30; 31; 32; 33; 34; 35; 36].

Ozone does not accumulate in plant organs, but can cause visible leaf injury (necrosis). For that reason, the leaf injury of sensitive plants can be used for assessing the effects of ozone [37; 38; 39; 40; 41; 42; 43; 44].

The origins of tobacco cultivars for biomonitoring are described in detail by [45]. They arose as a result of research initiated in 1957 to identify the cause of "weather fleck" in the USA – a mysterious disease which followed periods of hot sunny weather and devastated tobacco crops due to the appearance of extensive foliar lesions. Subsequently it was identified that ground-level ozone was the cause. During the course of a programme of breeding resistance into tobacco a supersensitive individual was identified from which the response indicator cultivar Bel-W3 was developed. In a similar manner the less sensitive Bel-C and tolerant Bel-B were developed. In Europe studies with Bel-W3 commenced in the late 1960s to early 1970s in the UK, Federal Republic of Germany, Belgium and the Netherlands [46; 47; 48; 49; 50].

The extent of the ozone-caused injury to the response indicator plant depends on the ozone dose absorbed. This is partly associated with the ozone concentration measured in the ambient air. High ozone concentrations are usually associated with high temperatures and low relative air humidity which can induce stomatal closure thereby decreasing the absorbed ozone dose. Moreover, high wind speed also decreases the concentration gradient between the ambient air and leaf surface thereby increasing ozone uptake. The tobacco exposure provides a direct measure of the impact of ozone on plants.

Significant relationships between the variables of ozone concentration or dose and ozone-induced leaf injury (=bioindicator response) in some species (e.g. wild and cultivated tomato species) have been reported by [51] and [52]. Ozone-induced injury on the extremely sensitive tobacco cultivar Bel-W3, however, cannot directly be translated into impact on native vegetation or crops. Nevertheless, leaf injury in tobacco Bel-W3 can be used as an indicator of the potential vegetation injury, i.e. the maximum vegetation injury to be expected under given pollution and climate conditions [53].

Since 2000, many investigations have employed widespread biomonitoring with Bel-W3 [54; 55; 56; 57; 58; 59; 60]. The largest international survey in Europe was conducted under the auspices of the EuroBionet-programme involving 12 cities in eight countries [61].

1 Scope

This European Standard applies to the determination of the impact of ground-level ozone on a bioindicator plant species (tobacco *Nicotiana tabacum* cultivars Bel-W3, Bel-B and Bel-C) in a given environment.

The present document specifies the procedure for setting-up and use of a system designed to expose these plants to ambient air. It also describes the procedure for leaf injury assessment.

Leaf injury caused by ozone appears in the form of necrosis or accelerated aging (senescence) on the leaves of the bioindicator. The macroscopically detectable leaf injury is used as the effect measure (see pictures in Annex A). The measure is the percentage of dead leaf area on the entire leaf surface.

The results of the standardized tobacco exposure indicate ozone-caused injury of the exposed bioindicators and thus enable a spatial and temporal distribution of the impact of ozone on plants to be determined.

This Standard applies to the outside atmosphere in all environments. This standard does not apply to the assessment of air quality inside buildings.

The method described in this European Standard does not replace modelling or physico-chemical methods of direct measurement of air pollutants, it complements them by demonstrating the biological effect.

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.

Note 2 to entry: Active biomonitoring refers to deliberate field exposure following a standardized methodology; passive biomonitoring refers to *in situ*-sampling and/or observation of selected bioindicators currently or previously present in the environment.

[SOURCE: EN 16413:2014 [62], 2.1, modified, note 2 to entry added]

2.2
bioindicator

organism or part of an organism or an organism community (biocoenosis) which documents environmental impacts

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

[SOURCE: EN 16413:2014 [62], 2.2, modified]

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

[SOURCE: EN 16413:2014 [62], 2.3]

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

[SOURCE: EN 16413:2014 [62], 2.4]

2.5
ground-level ozone
ozone present in the terrestrial biosphere

2.6
leaf necrosis
death of cells or tissues through injury or disease, especially in a localized area of the leaf

2.7
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.

[SOURCE: EN 16413:2014 [62], 2.9]

2.8
visual injury assessment
process of estimating the extent of macroscopically visible injury of the leaf surface

3 Principle of the method

The standardized method describes:

— the exposure of tobacco plants (cultivars Bel-W3 or Bel-C, and Bel-B) to the ambient air; and

— the assessment of the injury caused to the foliage by ground-level ozone.

The cultivar Bel-B is more tolerant to ozone pollution than Bel-W3 and Bel-C. It is used as a control to avoid confounding symptoms due to ozone with symptoms resulting from other factors (diseases in particular), to which all three cultivars are equally sensitive.

In areas where ozone pollution is expected to be particularly severe, the cultivar Bel-W3 can be too sensitive and can exhibit complete leaf damage. In this case it is better to use the cultivar Bel-C, which is less sensitive to ozone.

The repeated exposure of tobacco on several sites enables determination of the temporal and spatial distribution of ozone effects.

4 Test method

4.1 Material

4.1.1 Plants

Tobacco (*Nicotiana tabacum* L.) seeds of cultivars Bel-W3, Bel-C and Bel-B are used. Each study should be conducted with seeds derived from the same batch, as these cultivars can exhibit some degree of intra-cultivar variability in their response to ozone. Tobacco seeds can lose their viability over a period of a few years.

4.1.2 Substrate

For the cultivation and exposure, a light standardized soil is used. It is important to specify the nutrient content of the soil as this (in particular nitrogen) can modify the response of plants to ozone. Thus the substrate shall contain a basic nitrogen-phosphorus-potassium-content. The range of nutrients is N 200-300 mg/l; P_2O_5 250-350 mg/l; K_2O 300 −600 mg/l.

NOTE The NPK-content of commercial potting soils is frequently given as weight per litre of the product.

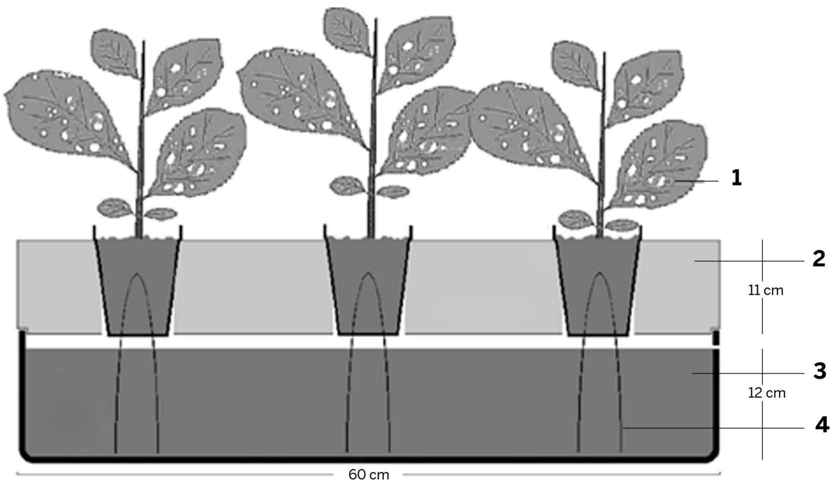
As such, further fertilisation during cultivation and exposure of the bioindicator plants is not necessary. The substrate should have a pH between 5,5 and 6,5. Before putting the soil into the plant pots, it should be moistened if necessary.

4.1.3 Water

For watering the plants drinking water quality (Council Directive 98/83/EC on the quality of water intended for human consumption [63]) is sufficient. If the values given there cannot be complied with, deionised water shall be used.

4.1.4 Exposure device

The exposure of the bioindicators takes place in commercially available square plastic plant pots with the dimensions 13 cm × 13 cm (top rim) and a height of 13 cm (volume: ca. 1,25 l to 1,5 l; see Figure 1) or in round pots with comparable soil volume. Four holes are drilled into the base of the pots (if not already present in the purchased pots), through which two moistened glass fibre wicks (diameter: 5 mm to 6 mm, length: 50 cm to 70 cm) or other suitable suction wicks are inserted. The wicks serve as a continuous water supply during cultivation and exposure. At least 7 cm of the wicks should reach into the substrate. The length of loose ends should be chosen in such a way that both ends reach the bottom of the water storage container. As water storage container, a Euro standard stackable plastic container (60 cm × 40 cm × 12 cm) is used, into which an overflow is drilled approximately 2 cm below the upper edge. A white polystyrene block (60 cm × 40 cm × 11 cm) with at least two recesses (11,5 cm × 11,5 cm) into which the plant pots are put is placed onto this tub. In this way, mutual obstruction/shading of the growing plants is avoided. Suitable shaping of its lower edge prevents the block slipping off the tub (Figure 1). For the plants, wooden or bamboo sticks are used as support to prevent wind damage.



Key

1: leaf with necrosis

2: polystyrene block

3: water reservoir

4: suction wick

Figure 1 — Example of a device for the exposure of tobacco plants

4.1.5 Exposure rack

The exposure rack consists of a solid frame construction (Figure 2). The tobacco plants are exposed at a height of 70 cm to 110 cm from ground level to the soil surface in the pots.

During the exposure water is supplied by the wicks, which hang from the plant pots into the water reservoir. A filling quantity of 20 l enables two weeks of maintenance-free exposure.

The exposure rack is covered with green shading fabric (shading rate 50 %) at the top and at three sides (east, south, west). It is open toward the north. The shaded plants react more sensitively to ground-level ozone than those under direct sunlight as the stomata of the leaves – being the dominating uptake path for ozone – remain open for longer periods of time. One can therefore expect a higher level of leaf injury in shaded plants.