SLOVENSKI STANDARD
oSIST prEN 16789:2014

01-november-2014

Zunanji zrak - Biomonitoring z višjimi rastlinami - Metoda izpostavljenosti standardnemu tobaku

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

Ta slovenski standard je istoveten z: **prEN 16789**

ICS:

13.040.20 Kakovost okoljskega zraka Ambient atmospheres

oSIST prEN 16789:2014 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 16789

September 2014

ICS 13.020.40; 13.040.20

English Version

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

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

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

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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. prEN 16789:2014 E

Contents

Foreword

This document (prEN 16789:2014) has been prepared by Technical Committee CEN/TC 264 “Air quality”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

Introduction

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].

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 Habitat 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“.

Development of the standardised 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 [17; 18; 19; 20; 21; 22; 23; 24]. Ground-level ozone also contributes to the development of forest decline [25; 26; 27; 28]. Effects of ozone on wild plants are the subject of numerous investigations [e.g. 29; 30; 31; 32; 33; 34; 35; 36; 37].

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 [38; 39; 40; 41; 42; 43; 44; 45].

The origins of biomonitoring tobacco cultivars are described in detail by [46]. 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 [47; 48; 49; 50; 51].

The extent of the ozone-caused injury of the response indicator plant depends on the absorbed ozone dose. 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. As such, the tobacco exposure provides a direct measure of the impact of ozone on plants.

Significant relationships between the bioindicator response and ozone-induced leaf injury in some species (e.g., wild and cultivated tomato species) have been reported by [52] and [53]. Ozone-induced injury on the extremely sensitive tobacco cultivar Bel-W3, however, cannot directly be translated into impact on native vegetation or crops. However, 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 [54].

Since 2000, many investigations have employed widespread biomonitoring with Bel-W3 [55; 56; 57; 58; 59; 60; 61]. The largest international survey in Europe was conducted under the auspices of the EuroBionet-programme involving twelve cities in eight countries [62].

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

Leaf injury caused by ozone appears in the form of necrosis or accelerated leaf 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 standardised 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 but 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 under standardised conditions; passive biomonitoring refers to in situ-sampling and/or observation of selected biological systems already present in the environment.

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
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.

3 Principle of the method

The method consists of exposing tobacco plants (cultivars Bel-W3 or Bel-C, and Bel-B) to the ambient air and quantifying the damage 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 the symptoms due to ozone, which are observed essentially on Bel-W3 and Bel-C, with symptoms resulting from other environmental stresses (diseases in particular), which are observed on all three cultivars.

In areas where ozone pollution is expected to be particularly severe, the cultivar Bel-W3 may be too sensitive and 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 the 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. Supplier information is provided in Annex B. Each study should be conducted with seeds derived from the same batch, as these cultivars may exhibit some degree of intra-cultivar variability in their response to ozone. Tobacco seeds may lose their viability over a period of a few years.

4.1.2 Substrate

For the cultivation and exposure, a light potting 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 the automatic water supply during the 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 stacking crate (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 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.

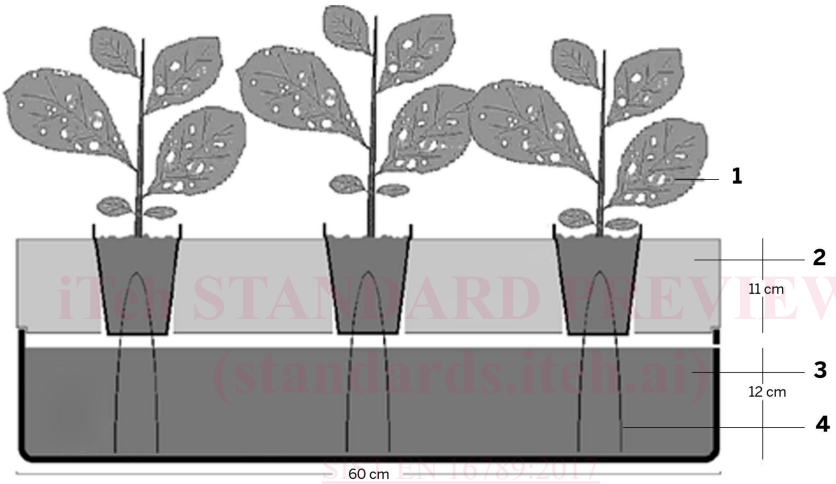

Figure 1 — Device for the exposure of tobacco plants

Key

1: necrosis

2: polystyrene block

3: water reservoir

4: suctionwick

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 the water is supplied by the wicks, which hang from the plant pots into the water reservoir. A filling quantity of 20 l ensures a fortnightly, 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 – as the dominating uptake path for ozone – remain opened for longer. One can therefore expect a higher level of leaf injury in shaded plants.

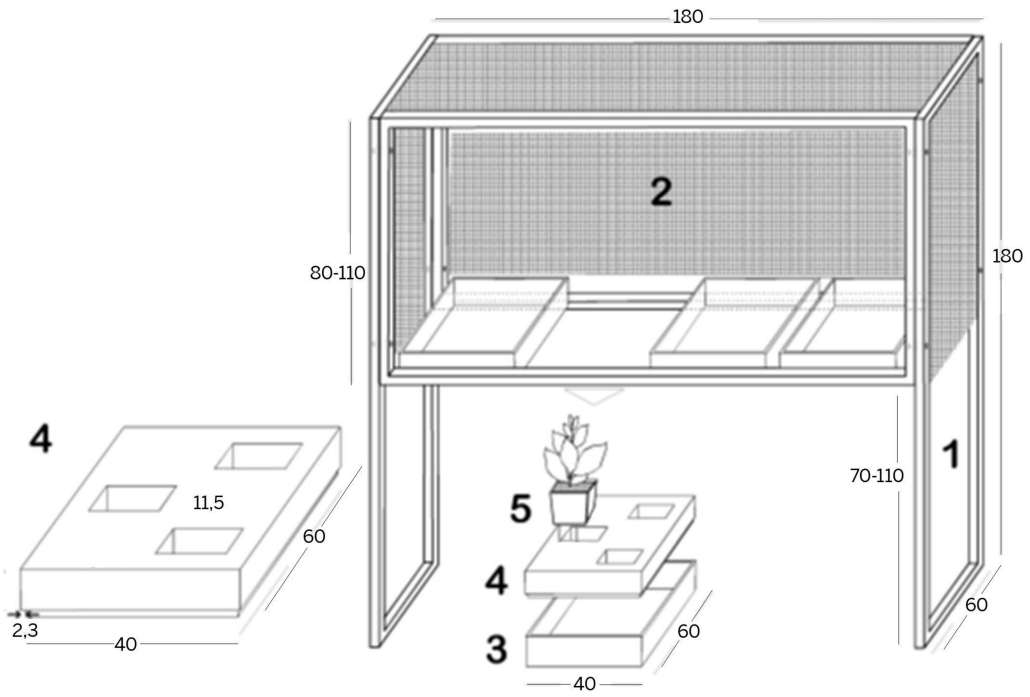

Figure 2 — Exposure rack (according to Arndt et al., 1985)

Key:

1: metal frame construction, consisting of four frame elements (2 pieces 180 × 90 cm, 2 pieces 180 × 60 cm)

2: shading fabric at three sides (east, south, west) as well as at the top

3: plastic piling crates (60 cm × 40 cm × 12 cm) as water storage containers

4: polystyrene block (60 cm × 40 cm × 11 cm) with two complete pot-shaped holes (11,5 × 11,5 cm) and raised edge to prevent slipping

5: plastic pot (13 cm × 13 cm) with suction wicks

4.2 Cultivation of plants

Aim of the cultivation is a healthy, vigorous plant.

The tobacco plants are cultivated in an environment which minimises ambient ozone concentrations (e.g. greenhouse, open-top chamber, phytotron, if possible supplied with charcoal-filtered air). Efforts should be made to ensure as constant temperature as possible during cultivation. At high temperatures the seedlings should be watered from top down using a sprinkler, in order to avoid overheating; at night the temperature should not drop below 10 °C. The aim is to produce plants at a comparable stage of development and thus similar sensitivity for all exposure periods.

The tobacco is sown in trays with well-moistened standardized soil (see Subclause 4.1.2). The top layer consists of sieved soil, which is smoothed to a flat surface. The seeds are applied evenly and spread very thinly (1 seed/cm^2 to 5 seeds/cm^2). Since tobacco plants require light for germination, the seeds are not covered with soil. The seed trays are maintained at temperatures between 20 °C to 25 °C, in order to ensure a safe and rapid germination after four to six days (at temperatures of 18 °C to 20 °C it can take up to ten days before the seeds germinate). During this time, the surface of the soil should be kept moist (e.g. transparent cover on the tray).

Unused seeds are stored in a refrigerator (4 °C to 6 °C) for up to two years.

After the initial growth, the seedlings (see Figure 3a) should be transferred to a cooler place (approx. 18 °C to 20 °C) with ample light.

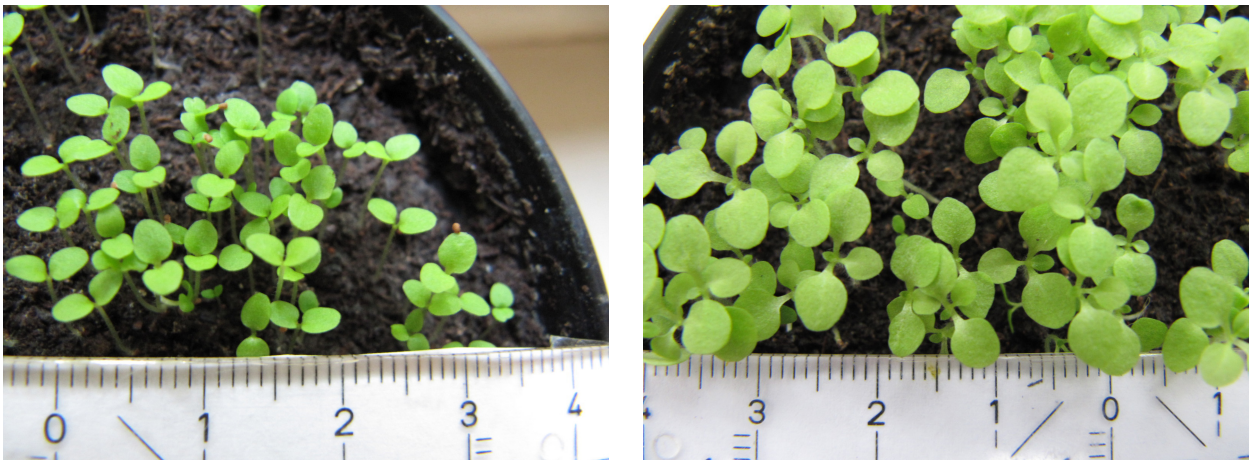

Figure 3 — a) Seedlings **b) Two leaf-stage**

The following cultivation procedure is recommended: Two to three weeks after sowing, the seedling reaches the two leaf-stage (two successive leaves after the two cotyledons; the latter do not count as leaves; see Figure 3b). At this stage, four small bunches of two to six seedlings each are taken from the sowing trays and transferred into the exposure pots (Figure 4).

Figure 4 — Tobacco seedlings, immediately after potting

The plants are watered carefully the first time using a thin spout to avoid damage to the seedlings. The pots are put into a water storage container without the polystyrene block and set up in a protected, relatively shaded place. The bottom of each container is covered with some water, so that the soil in the pots always remains moist by the use of the suction wicks. In the next two to three days it is checked whether at least one plant from each of the four bunches has established (areas where no plant has established satisfactorily should be replanted). One week after the replanting at the latest, four seedlings out of the bunches are selected in such a way that in each pot remain four plants of different sizes (Figure 5).