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BSI Standards Publication

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

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National foreword

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

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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of an identical text or by endorsement, at the latest by August 2014, and conflicting national standards shall be withdrawn at the latest by August 2014.

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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]).

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^2, 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

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.

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

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.

6.2 Moss species

In the framework of an air biomonitoring study, all terrestrial moss species (pleurocarpous and acrocarpous mosses) present in the environment can be sampled. The choice is guided by various criteria, including:

— **Ubiquity**: for studies conducted over very large areas, the widespread presence of the chosen species should be as great as possible.

— **Abundance**: the chosen species shall be sufficiently abundant in the study area to preserve the population durability and provide enough biomass for the chemical analyses.

— **Protection status**: the chosen species shall not be a protected species (IUCN Red List, species of Natura 2000 network, or on any other national or regional list of protection) to avoid the destruction of populations. The operator shall review these lists before beginning the study.

— **Identification**: the chosen species should be easy to identify in the field with minimal logistic means. Sampling requires that personnel have the necessary expertise. However, it is necessary to ensure in the laboratory that the species were correctly identified.

NOTE A list of the moss species most commonly used in bioaccumulation studies is given in Annex C. The operator will be in a position to compare his results to literature data (see Bibliography).

6.3 Period of collection

The duration of the period of collection shall be as short as possible. Weather conditions should be relatively constant during the sampling period and specific meteorological events (e.g. rain, snow) shall be avoided. Weather conditions just before and during sampling shall be recorded on the survey sheet.

The time of year for sampling to be carried out is important and needs to be determined accurately. In the case of temporal monitoring surveys which involve repeated measurements in time, it is important to investigate the same sampling units at the same period of the year. If not, precautions shall be taken in order to enable comparison of the data obtained in the same areas but at different times. Indeed, seasonal differences in concentrations of various elements in mosses have been reported in the literature ([22], [23], [24]). Seasonal variations can be attributed to a dilution effect, due to biomass increase during wet periods of moss growth, or to the loss of elements in the summer, due to desiccation effects and rapid rehydration ([25]).

In dry and barren environments, moss samples can be contaminated by high levels of windblown dust from local soils. In Mediterranean countries, for example, levels of airborne soil dust may be very high in summer and precautions shall be taken in order to gather comparable data for moss samples from different environments or for those collected in the same area, but at different times ([21]).

6.4 Sampling unit characteristics

Terrestrial mosses colonize various environments (including forests, moorlands, obsolete industrial sites and urban environments) and can grow on different substrata (mineral supports, humus and wood). In order to monitor air pollution only and to minimize the influence of other factors, the following characteristics of the sampling units shall be respected:

— Mosses shall be collected in open areas; no obstacle shall intercept or modify the direct atmospheric deposition on mosses. For example, in woodland, sampling shall be carried out preferentially in open spaces outside the canopy of trees, bushes or high forbs; in urban areas, samples shall not be taken from under roof eaves. Mosses shall not be collected in the proximity of high-voltage power lines due to a possible influence on metal contents.

— The surface of the substrate for the subsample collection should be as level as possible (< 30°) to avoid enrichment of the deposition due to the effect of a slope. In the case of inclined surfaces, it is preferable to undertake the sampling at the top of the slope to avoid the pathway of any runoff. Vertical surfaces

shall be avoided (e.g. tree trunks, embankments, roofs, walls). Sampling from partly flooded environments shall be excluded because of the deposition.

— To allow comparisons between sites, mosses should have an identical substrate in all different sampling units (e.g. rock, stump, concrete).

7 Sampling procedure

7.1 General

In each sampling unit, the moss sample shall be composed of material of only one species. Nevertheless, because moss carpets usually overlap with the substrate and hence other species, a complete cleaning of the material to obtain a monospecific sample is not always possible in the field. During the field work, operators shall concentrate as much as possible on the target species to facilitate the final cleaning in the laboratory.

7.2 Moss sample

The moss samples which will be analyzed shall be monospecific to avoid variations in concentrations between samples, due to intrinsic differences between species. A specific inter-species comparison is recommended when different moss species are collected during the same monitoring survey (see 10.2.2).

The moss sample shall be representative of the sampling unit. The moss sample shall be composed of several sub-samples (≥ 10) evenly distributed in the target population within the sampling unit. Sub-samples should be collected according to a probabilistic sampling strategy to reduce the effects of local variability (due to inter-individual differences and deposition processes) on the results ([26], [27]). The collected subsamples shall be combined to create a composite sample which is representative of the sampling unit.

7.3 Sample collection

Moss samples are to be collected, handled and transported in such a way that their contaminant concentrations remain unchanged.

The materials that come into contact with the moss samples shall be designed so as not to interfere with the contaminants to be analyzed. The use of disposable materials is strongly recommended (e.g. non-talc gloves and containers). If materials are re-used, they shall be cleaned between successive uses and the washing protocol shall be set according to the contaminants to be assayed and the materials used. All additional sources of contamination (e.g. smoking) shall be avoided during sampling.

The recommended quantity of cleaned moss samples for analysis and storage purposes is about 5 g of dry weight for one moss sample. However, this quantity shall be adapted according to the requirements of the analysis laboratory, relative to the number and nature of analysed elements. The operator shall find out the equivalence of this quantity in fresh weight and volume before the beginning of the study.

Subsamples shall be picked over and put side by side (green living parts in contact) on top of each other in a container, carefully closed to prevent contamination during transportation. All the necessary identification data should be indicated on this container (e.g. sampling unit code, collection place and date). The final cleaning should be done in the laboratory.

If the final cleaning cannot be done in the laboratory or for specific compound analyses, moss samples shall be picked over shoot by shoot and cleaned directly in the field.

7.4 Packing

The moss samples shall be packed in inert pre-labelled containers. If the samples collected are damp they shall be dried rapidly at room temperature (< 35 °C) with filtered ambient air before cleaning. Once adopted,

the procedure for drying shall not be changed as air drying at room temperature on a laboratory bench or in a ventilated hood could influence the concentration of semi-volatile elements (e.g. Hg) ([28]).

To prevent loss of volatile organic contaminants, package the collected moss samples in pre-labelled aluminium tubs or coloured glass recipients. Maintain the collected moss samples at a temperature below 4 °C, clean and send them for analysis as rapidly as possible (within 24 h). If it is not possible, samples shall be frozen as promptly as possible after sampling and cleaning (temperature at −18 °C ± 2 °C).

8 Sample preparation

8.1 Sample cleaning

The material should not be touched with bare hands. Moss samples should be placed on a bench with an inert material surface (e.g. plastic food film) and sorted to remove all plant, animal and mineral debris adhering to the moss shoots. Particular attention shall be given to minimize the effect of soil contamination (particulate material deposited on the moss surface). The samples shall not be washed. A digital macro full frame photo should be taken from the total moss material before cleaning in order to be able to clarify any subsequent ambiguities (species, contaminations, etc.).

The part of the moss to be sampled can have an effect on the interpretation of the analytical data, due to the fact that elements are not evenly distributed along the moss shoot ([24], [29], [30]). Hence, great care is required to process all moss samples in the survey in the same way, keeping either the whole moss shoot, the green apical part or a fixed length of the moss shoot.

8.2 Sample homogenization

The moss samples shall be ground after drying and cleaning. Different grinding equipment can be used but care shall be taken to use contaminant-free grinding materials, which shall be thoroughly cleaned between two samples.

8.3 Sample storage

For non-volatile contaminants, storage can be considered but it is important that the method and duration of storage does not influence the contaminant concentration. Samples shall be stored away from heat, moisture and light to avoid degradation and changes in the substances of interest.

In the case of volatile contaminants, moss samples shall be analysed as soon as possible after cleaning.

9 Recommendations for sample analysis

The contaminant determinations can be performed using various analytical techniques associated or not with a method of digestion. The analysis should be performed by an accredited laboratory, qualified and approved for the respective analytical method. The laboratory should have experience with complex matrices and is also obliged to apply the appropriate quality assurance measures (e.g. the analysis of certified standards).

The contaminant concentration value should be expressed as the dry weight of mosses at 105 °C ± 2 °C.

10 Quality Control and Quality Assurance

10.1 General

Proper Quality Assurance (QA) and Quality Control (QC) procedures should be implemented to control errors and document the overall quality of the monitoring survey. These procedures form an integral part of the study design and results. Studies failing to report their QA procedures and Quality Control results shall be regarded as incomplete.

10.2 Quality Control

10.2.1 Overall variability

The overall variability associated with the entire procedure, from sampling to moss analysis, shall be characterized. This means that, in at least two sampling units of the study area (where the highest and the lowest contamination levels, respectively, are expected), a multiple moss sampling (number of samples ≥ 3) shall be carried out. The moss samples shall be collected (for each composite sample, there shall be 10 or more subsamples as described in 7.2), transported, stored, processed in the laboratory (as described in 7 and 8) and analysed separately. A relative standard deviation (RSD) shall be calculated to characterize the total variability of the data.

10.2.2 Interspecies calibration

A specific inter-species comparison is recommended when different moss species are used for the same biomonitoring study of atmospheric contaminants. Different moss species should be collected (as described in Clause 7) at the same sampling unit in order to compare their contaminant levels and identify correlations. However, it is best not to convert data for one species into data for another as this can increase uncertainty in the results.

10.2.3 Storage of the samples

During the cleaning stage of each moss sample, a aliquot composed of several whole dried moss shoots shall be stored for verification of the moss species determination. These sub-samples and also the ground moss samples shall be kept until three months after publication of the final report.

10.3 Quality Assurance

Each investigation shall be completely and thoroughly documented (operating procedure adopted) and the documentation shall be included in the report, so that an evaluation of the results and a repetition of the study are possible. Each monitoring study shall present the following basic data:

— description of the sampling design adopted and its justification in relation to the study objectives;

— description of the method to be used to identify the sample location on maps and in the field;

— description of data collection methods in the field, including equipment and field forms;

— description of field conditions (weather, moss species, geographical coordinates…);

— description of the chain of custody of the data (recording in the field, storage in electronic format, transmission to central database – if any).

Annex A
(informative)

Example of a survey sheet

The following data should be collected:

— date of sampling;

— sampling unit code;

— coordinates of the sampling unit;

— altitude;

— locality (region, municipality, etc.);

— weather (raining, sunny, cloudy, foggy);

— moss species sampled;

— land use (for example, a precise level of the Corine land cover classification);

— image of the sampling unit;

— characteristics of the moss substrate (e.g. rock, dead wood, concrete);

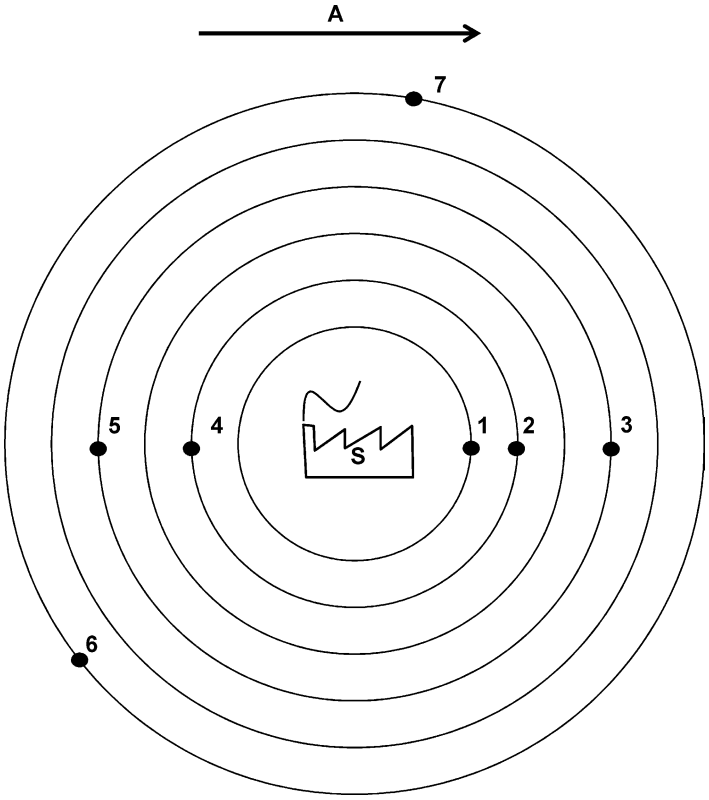
— minimal distance from the nearest tree, shrub or herbaceous canopy;

— distance from the monitored and other possible emission sources;

— distance from main roads, to residential and agricultural areas.

Annex B
(informative)

Example of the location of the sampling units near an emission source



Key

A main direction of the wind

S source of emission

1 to 7 monitoring units

Figure B.1

To assess the impact that an emission source can have on its vicinity, at least seven monitoring sites are needed (this is an optimal situation):

— at least three sampling units (nos. 1, 2, 3) should be located on a straight line, running from the emission source along the most frequent wind direction;

— at least two sampling units (nos. 4, 5) should be located on a straight line, running from the emission source along the least frequent wind direction;

— at least two sampling units (nos. 6, 7) are intended to assess the background level of contaminants in the monitoring area; sites shall be located far from all known sources of isolated pollution.

NOTE Wind direction is that towards which the wind is blowing.

Annex C
(informative)

List of main moss species used in published bioaccumulation studies

Abietinella abietina (Hedw.) Fleisch.
Atrichum undulatum (Hedw.) P. Beauv.
Brachythecium rutabulum (Hedw.) Schimp.
Bryum argenteum Hedw.
Bryum radiculosum Brid.
Ceratodon purpureus (Hedw.) Brid.
Dicranella heteromalla (Hedw.) Schimp.
Dicranoweisia cirrata (Hedw.) Lindb.
Dicranum polysetum Sw. ex anon.
Dicranum scoparium Hedw.
Funaria hygrometrica Hedw.
Grimmia pulvinata (Hedw.) Sm.
Homalothecium lutescens (Hedw.) Robins
Homalothecium sericeum (Hedw.) Schimp.
Hylocomium splendens (Hedw.) Schimp.
Hypnum cupressiforme Hedw.
Leskea polycarpa Hedw.
Mnium hornum Hedw.
Orthotrichum anomalum Hedw
Plagiothecium denticulatum (Hedw.) Schimp.
Plagiothecium undulatum (Hedw.) Schimp.
Pleurozium schreberi (Willd. ex Brid.) Mitt.
Pohlia nutans (Hedw.) Lindb.
Polytrichastrum formosum (Hedw.) G.L.Sm.
Polytrichum juniperinum Hedw.
Pseudoscleropodium purum (Hedw.) M.Fleisch.
Rhacomitrium lanuginosum (Hedw.) Brid.
Rhytidiadelphus squarrosus (Hedw.) Warnst.
Rhytidiadelphus triquetrus (Hedw.) Warnst.
Rhytidium rugosum (Hedw.) Kindb.
Scleropodium touretii (Brid.) L.F.Koch
Syntrichia ruralis (Hedw.) F.Weber and D.Mohr
Thuidium delicatulum (Hedw.) Schimp.
Thuidium tamariscinum (Hedw.) Schimp.
Tortula muralis Hedw.

Nomenclature of the moss taxa follows *Hill* et al. (2006) ([31]).

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[6] Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe

[7] Directive 2008/1/EC of the European Parliament and of the Council of 15 January 2008 concerning integrated pollution prevention and control

[8] Directive 2010/75/EU of the European Parliament and of the Council of 24 November 2010 on industrial emissions (integrated pollution prevention and control)

[9] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

[10] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed - Council statement

[11] Commission Directive 2005/87/EC of 5 December 2005 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards lead, fluorine and cadmium

[12] Commission Directive 2006/13/EC of 3 February 2006 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs

[13] Directive 2004/107/EC of the European Parliament and of the Council of 15 December 2004 relating to arsenic, cadmium, mercury, nickel and polycyclic aromatic hydrocarbons in ambient air

[14] Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora

[15] COM/2003/0338 final Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee - A European Environment and Health Strategy

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