UMWELTPROBENBANK DES BUNDES

Guideline for Sampling and Sample Processing

Red Beech (*Fagus sylvatica*)

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Contents

Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

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1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) subject to specialist and administrative coordination by the Federal Environment Agency (UBA). The ESB collects ecologically representative environmental and human samples and stores and investigates them for environmentally relevant substances.

Specific operating procedures as well as the conception of the ESB are the basis of the program. (Umweltbundesamt 2008, 2014)

The long-term storage is carried out under conditions which, as much as possible, exclude a change in state or a loss of chemical characteristics over a period of several decades. The archive therefore provides samples for retrospective investigations of substances for which the potential risk for the environment or human health is not yet known.

Comprehensive information on the ESB is available at www.umweltprobenbank.de.

2 Objective of this Guideline

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based, standardized methods to avoid contamination and inhibit loss of chemical information. The need for an exceptionally high level of quality assurance results from the extraordinary value of the samples as archive material. Representativeness and reproducibility of the samples are the basis for spatial and temporal comparison.

The current guideline is an update of the Wagner *et al*. (2009) version.

Transport, further sample treatment and storage as well as chemical analysis have to be carried out according to the current guidelines of the ESB.

The coordination of the sampling guideline with the VDI (The Association of German Engineers) Standard 3957 Blatt 11 (VDI 2007), guarantees the comparability of the test results with other monitoring programs.

3 Function of the Specimen Type

The leaves of the summer green deciduous trees are only exposed to environmental influences during the vegetation period. Thus they are indicators for characterizing effects of air-borne pollution during the vegetation period. They are used complementary to evergreen conifers.

The European Red Beech (*Fagus sylvatica L*) is found throughout Central and Western Europe where it is the most important and native deciduous tree. The natural distribution of the red beech is between Belarus and the Baltic to the Cantabrian Mountains of Northern Spain. The North-South distribution ranges from Southern England and Southern Sweden to the mountains of Sicily and the Balkans. The proportion of beech in Germany is approximately 15 % of the total forest area of 11.4 million ha (BMEL 2012).

The red beech is suitable for the ESB because of its importance as a primary producer and key species in numerous, close-to-nature and anthropogenically-influenced ecosystems throughout Central Europe. Due to their size and structure, free-standing beeches are particularly exposed to and impacted by air pollution and they act as a filter to flowing air.

The following criteria underline the appropriateness of the use of the red beech for the ESB:

- availability of comprehensive baseline and comparative data (BMELF 2000, Fischer and Lorenz 2000, BMVEL 2004),
- physiologically and eco-physiologically well studied species (Masarovičová *et al*. 1999, Neirynck and Roskams 1999, Bortier *et al.* 2000, Günthardt-Georg *et al*. 2000, Schraml and Renneberg 2000, Tarricone *et al.* 2015),
- usage of the beech as an indicator organism since the early 1960s (i.a. Guderian and Stratmann 1968, Zimmermann 1986, 1989),

- continuous leaf exposure, thus the leaf pollutant content represents the total pollution per vegetation period with a single annual sampling.

4 Target Compartments

In accordance with the ESB, leaves without stalks are collected as target compartments. Beech leaves are even and remain free of any wax covering during their entire growth period. During their growth leaves are coated with fine hairs, which rapidly dwindle through abrasion. Subsequently a homogeneous even leaf surface remains (Neinhuis and Barthlott 1998).

5 Predefinitions for Sampling

5.1 Selection and Definition of Sampling Sites

In order to determine the sampling site(s) and sampling size, a screening according to the stratified random sampling principle (Green 1979) must be carried out before the first sampling in a sampling area.

In the first step, homogeneous screening areas in the sampling area are determined with regard to the following criteria, for example:

- geology
- soil
- exposure
- gradient of slope
- height
- sufficient distance from busy roads and other local emitters
- availability of beech stands that are at least 40 years old.

Care must be taken to ensure that the selected stands are also suitable long-term locations from the point of view of forest planning and forest protection.

An appropriate number of screening areas shall then be determined at random, on which at least 30 trees should be examined. At least three (preferably six) trees should be selected from each area.

After the chemical characterization analysis has been carried out, the dispersion range of the pollutant contents and the spatial pattern of the pollution burden are examined. On the basis of these results, the sampling site is determined as the sum of the suitable screening sites (= future sampling points).

Access to the designated sampling points should, as much as possible, be secured by contracts.

5.2 Selection of Individuals and Sample Size

After evaluation of the screening results, the sample size for the annual routine sampling is determined. The minimum sample size is 15 trees per sampling site.

For a sample collective of 15 trees, a minimum of 75 g fresh weight (leaves without stalks) per tree should be collected, in order to adequately represent the respective tree and to reach the required total sampling quantity of 1,100 g.

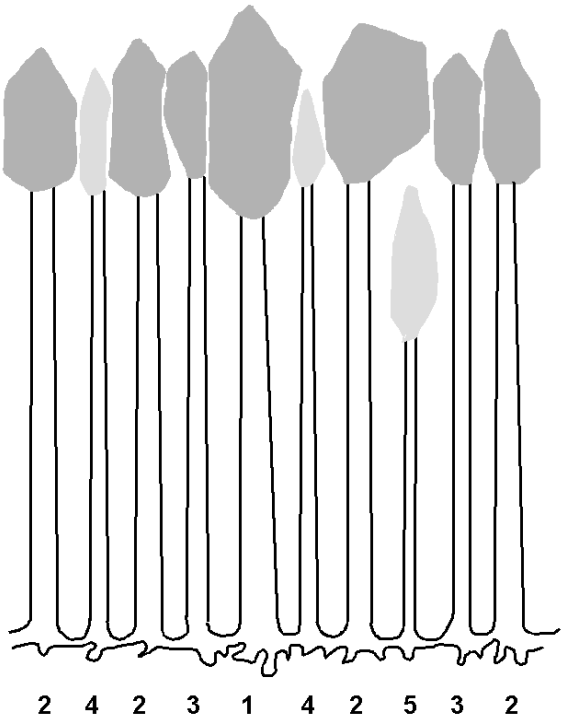

Fig. 1: Tree Categories by KRAFT (1884) (1 = pre-dominant, 2 = dominant, 3 = co-dominant, 4 = dominated, 5 = complete epigenous (from BMJ 2000))

The trees are randomly selected within the locations and should comply with the following criteria:

- more than 40 years old, predominant, dominant or co-dominant (Fig. 1),
- free from intense biological (e.g. feeding on leaves) or mechanical damage.

5.3 Sampling Period and Frequency

In long-term programs such as the ESB, sampling should be carried out annually.

Sampling should take place in the late summer before leaf discoloration. Since this period varies both annually and in dependence with the climate zone, varying sampling periods for different sampling areas result. The sampling should be completed in the lower elevation areas by the end of August and in higher elevation areas by mid of September.

5.4 Area Related Sampling Scheme

Based on the sampling guidelines, specific definitions for the individual sampling areas and sites must be made and documented in an area related sampling scheme. These include, but are not limited to:

- location and demarcation of the sampling sites,
- required sample size,
- time frame for sampling,
- appropriate authorities (e.g. forestry offices).

Here it is important to consider how to ensure a long-term sampling continuity. If changes are made, the document must be updated.

6 Sampling Procedure

All data collected during sampling and biometric sample characterization must be documented in the corresponding specimen data sheets (see appendix). In addition, a protocol must be prepared for each sampling with the following information:

- persons that participated in the sampling,
- chronological sequence of the sampling,
- the underlying version of the sampling guideline and the area-related sampling scheme for the current sampling as well as,
- deviations from the sampling guideline and the area-related sampling scheme.

Collecting specimens from the crown area of standing trees is performed by and exclusively permitted by persons suitably qualified („cone pickers“) with respect to the safety regulations of the professional association. Their health condition also must be regularly checked. If special requirements exist, e.g. to protect the trees from which samples are taken against damage, appropriate tree climbing equipment has to be used.

6.1 Required Equipment and Cleaning Procedures

Field work:

- specimen data sheets,
- stainless steel scissors,
- stainless steel trough to catch the cut leaves,
- stainless steel containers (1.5, 3.5 or 5.5 l according to the amount being collected) with lids and fasteners,
- waterproof pen for labeling the paper bags and stainless steel containers,
- paper bags (1 bag per tree),
- disposable gloves,
- scale for checking the minimum sample quantity (weighing range up to at least 3 kg, reading 1 g),
- air thermometer,
- soil thermometer,
- camera for documentation,
- liquid nitrogen,
- protective clothing for liquid nitrogen handling,
- cooling device (dewar) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN), corresponding to the number of required stainless steel containers.

Laboratory:

- specimen data sheets for the biometric sample description,

- cabinet dryer (approx. 80°C),
- scale (reading 0.01 g),
- weighing pans,
- stainless steel tweezers.

Sample containers and all equipment is cleaned in a laboratory washer using a chlorine-free powerful washing agent in a first step. After cold and hot (90 – 95°C) rinsing, neutralization using 30% phosphorus acid in warm water is performed, followed by hot and cold rinsing with deionized water. After this procedure, the containers are dried in a cabinet dryer at 130°C (± 10°) for a minimum of an hour (sterilization). The containers remain in the closed cabinet dryer while they are left to cool. Sterilization is not applied to synthetic materials.

6.2 Sampling Technique

Sampling is only carried out in dry weather conditions and stopped if it starts raining. Morning dew must have evaporated on the leaves in the tree crown prior to starting or continuing the collection. Inevitable deviations must be precisely noted in the sampling record.

From each tree at least 3 branches that represent different exposure directions are sawed from the crown area. Generally, branches are taken from the upper crown area. If free-standing beech trees are sampled outside of closed forest areas, e.g. on urban or agricultural areas, sampling can be carried out from the ground in the woodland edge area using loping pole pruners. Damaged branches should not be used. The branches removed from the tree must not be too large and the bark should not be damaged.

If the tree is sufficiently far enough away from other trees, the branches can be thrown down, but they must not be contaminated when they hit the ground (e.g. due to wheel tracks or fresh ground damage caused by wild boars). If necessary, a storage area made of deadwood is to be prepared for the sample branches.

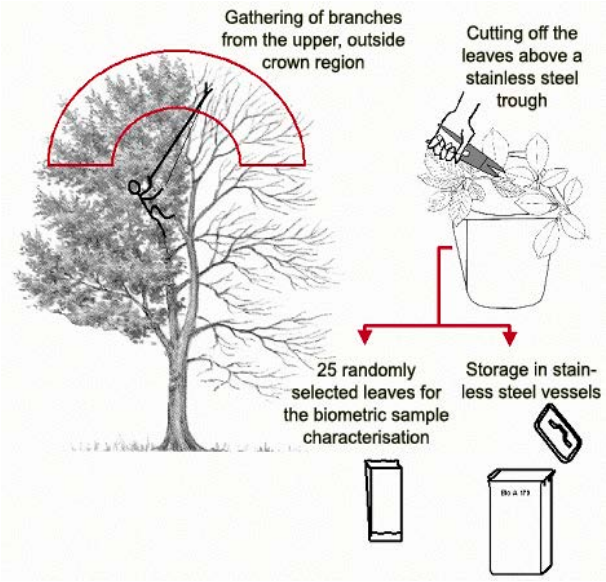

Fig. 2: Schematic representation of the sampling

After the sample characterization (chap. 7), the leaves without their stalks are cut directly and without touching into the stainless steel trough, using stainless steel scissors (Fig. 2).

For further sample characterization, 25 leaves per tree are randomly taken from the total sample and collected in a labeled paper bag.

The remaining leaves are transferred in the required quantities from the stainless steel trough into the storage containers, whose empty weight has previously been taken. During this procedure, laboratory gloves are to be worn.

The samples are immediately rapid-frozen on-site in a dewar vessel for the further storage and transportation of the samples in the gas phase above liquid nitrogen (LIN).

7 Biometric Sample Characterization

The parameters for sample characterization listed in specimen data sheet 3 are to be recorded in the field at the branches obtained before the leaves are cut off.

To determine the dry weight (reading to 0.01 g), the 25 randomly selected leaves in the paper bags are placed in a cabinet dryer (approx. 80°C)

immediately after returning from sampling and dried to constant weight (about two days) in the laboratory.

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Checklist to Prepare and Conduct the Sampling

Specimen Type	**Red Beech (*Fagus sylvatica* L.)**
Target Compartment	leaves without leaf stalks from at least three branches in the upper crown area that are exposed and receive sunlight
Individual Specimens	predominant, dominant or co-dominant trees (tree category 1, 2 or 3 by BMJ 2000), older than 40 years
Random Sample Number	at least 15 trees
Sample Quantity for the ESB	for a total sample mass of 1,100 g, 75 g fresh weight (= leaves without stalks) from 15 trees must be sampled
Sampling Period	in late summer prior to leaf discoloration until the end of August or middle of September (depending on climatic region)
Sampling Frequenzy	1 sampling per annum
Equipment Required for Field Work	• specimen data sheets • shears with a telescopic handle extendable to about 5 m • stainless steel scissors • stainless steel container (trough) to catch the cut leaves • waterproof pen to label the paper bags and stainless steel containers • disposable gloves • scale (weighing range up to at least 3 kg, reading 1 g) • air thermometer, soil thermometer • camera for documentation
Sample Packing until Further Processing	• stainless steel containers (1.5, 3.5 or 5.5 l) with lids and fasteners (1 container per tree), • paper bags (1 bag per tree)
Transport and Interim Storage	• cooling device (dewar) for rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN)
Required Equipment for Laboratory Work	• specimen data sheets for the biometric sample description • cabinet dryer (approx. 80°C) • precision scale (reading 0.01 g) • weighing pans • stainless steel tweezers
Sample Characterization	• stand type, tree height • fructification • damage (feeding on leaves, chlorosis, necrosis), contamination • for 25 leaves: dry weight of the leaves (reading 0.01 g)

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

Red Beech (*Fagus sylvatica)*

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

- Specimen Type
- Specimen Condition
- Collection Date (MM/JJ)
- Sampling Area (SA)
- Sampling Region (SR)
- Sampling Site (SS)
- Additional information

Sampling Site (plaintext) _____

Sampling Point (number) _____

Sampling Point (plaintext) _____

Sampling Leader _____

Remarks _____

Notes _____

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 2: Weather Conditions

Red Beech (*Fagus sylvatica*)

Identification:

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Numbers: from __ __ to __ __

Last Precipitation Date Preceding the Sampling: __ __ . __ __ . __ __ __ __

Type of Precipitation:
(see table below) ___

Start of the Sampling:		**End of the Sampling:**
__ __ . __ __ . __ __	Sampling Date	__ __ . __ __ . __ __
__ __ : __ __	Time	__ __ : __ __
__ __	Air Temperature at 1.5 m Height (°C)	__ __
__ __	Soil Temperature at 10 cm Depth (°C)	__ __
__ / 8	Cloud Covering	__ / 8
__	Type of Clouds (see table below)	__
__ __ __	Wind Direction	__ __ __
__	Wind Force in Degree Beaufort (see table below)	__
__	Type of Precipitation (see table below)	__

Type of Clouds:
0 = unclouded
1 = Cirrus
2 = Stratus
3 = Cumulus
4 = Fog
5 = high Fog
6 = Stratocumulus




Cirrus Stratus Cumulus Stratocumulus




Type of Precipitation:
0 = no Precipitation
1 = rain
2 = drizzle
3 = sow
4 = dew
5 = rime
6 = torrential rain
7 = hail

Wind Force (according to Beaufort):
0 = calm
1 = very slight breeze
2 = slight breeze, moves leaves
3 = light breeze, moves twigs
4 = moderate breeze, moves thin branches
5 = bright breeze, moves medium sized branches
6 = strong wind, moves thick branches
7 = stiff Wind, shakes trees

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 3: Description of the Tree
Red Beech (*Fagus sylvatica*)

Identification: __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Number: __ __

Stand Type:
- ☐ Dense Stand
- ☐ Sparse Stand
- ☐ Free Standing Solitary Trees
- ☐ Marginal Stand Zone
- ☐ Forest Aisle

Tree height (estimation in 5 m steps)**:** __ __ **m**

Location of Sampled Branches in Crown
- ☐ Upper Outer Crown (Normal Case)
- ☐ Upper Inner Crown
- ☐ Lower Inner Crown
- ☐ Lower Outer Crown

Damages at Leaves (regarding the upper side of the leaf, more than one type can be selected, percentage given >0 – 5 = 5%, >5 – 10 = 10% etc

Feeding on Leaves

__ __ __ %
(Percentage of the leaf surface, estimation at 5% intervals)

Damage Type
- ☐ Nonexistent
- ☐ Pitting
- ☐ Mining
- ☐ Leaf Skeletonizing
- ☐ Sucking Spots of Insects
- ☐ Other: _____

Chlorosis

__ __ __ %
(all yellowish to whitish discolorations, estimation at 5% intervals)

Chlorosis Type
- ☐ Nonexistent
- ☐ As Stippling
- ☐ Blotchy, Skewbald

Chlorosis Dissemination on Leaf
- ☐ Nonexistent
- ☐ in the Middle
- ☐ Tip Burn
- ☐ Marginal Scorch
- ☐ Interveinal
- ☐ At whole Leaves

Necrosis

__ __ __ %
(all brownish to reddish discolorations, estimation at 5% intervals)

Necrosis Type
- ☐ Nonexistent
- ☐ As Stippling
- ☐ Blotchy, Skewbald

Necrosis Dissemination on Leaf
- ☐ Nonexistent
- ☐ in the Middle
- ☐ Tip Burn
- ☐ Marginal Scorch
- ☐ Interveinal
- ☐ At whole Leaves

GERMAN ENVIRONMENTAL SPECIMEN BANK
Specimen Data Sheet 4: Sample Description and Storage
Red Beech (*Fagus sylvatica*)

Identification:
__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

Tree Number: __ __

Modification of or Overlay on Leaf Surface	**Type of Overlay**
Total regarding the **upper** side of the leaf __ __ __ % Total regarding the **under** side of the leaf __ __ __ % (percentage estimate of the affected leaf area in 5% intervals)	☐ Nonexistent ☐ Honeydew ☐ Sooty Mould ☐ Fungal Diseases on Leaf ☐ Gall Mite (Top Side) ☐ Gall Mite (Bottom Side) ☐ Gall Midges ☐ Other: _____ _____

Fruiting: ☐ none ☐ medium ☐ strong

Dry Weight of the Leaves: __ __ , __ __ g, related to 25 randomly selected leaves

Storage

Storage condition: ☐ Dry Samples (standard) ☐ Humid Samples

Number of Stainless Steel Vessel:	Weight Empty [g]	Weight Filled [g]	Weight Sample [g]	Remarks
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	

Remarks: _____

_____

Dry weight determined (date): Signature:

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Protocol

Red Beech (*Fagus sylvatica*)

Sampling Area: _____ Identification: __ __ __ __ __

Underlying Version of the Sampling Guideline __ __ . __ __ . __ __

Underlying Version of the Sampling Scheme __ __ . __ __ . __ __

1. Sampling Objective: _____

_____

2. Actual Timeframe of the Sampling:

Start		End		Sample no.		Sampling Leader	Remarks
date	time	date	time	from	to		

3. Participants: internal _____

_____

external _____

4. Checklist Referring to Sampling Scheme and Sampling Guideline: ☒ as prescribed

- ☐ 4.1 Sampling Period
- ☐ 4.2 Sampling Site and Sampling Point (selection/definition)
- ☐ 4.3 Selection of the Individual Specimens
- ☐ 4.4 Technical Preparations
- ☐ 4.5 Cleaning Procedure for the Packages
- ☐ 4.6 Sampling Technique/Method of Capture
- ☐ 4.7 Sample Amount
- ☐ 4.8 Data Collection
- ☐ 4.9. Transport and Interim Storage

Number, kind and reason for deviation (clear text):

_____

_____

_____

_____

Remarks: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature