UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Treatment

Red Beech (*Fagus sylvatica*)

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Für Mensch und Umwelt

Contents

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

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German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument of environmental monitoring for the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) underlying specialized and administrative co-ordination of the Federal Environmental Agency (Umweltbundesamt, UBA). The ESB collects ecologically representative environmental specimen in addition to human samples, maintains and examines them concerning relevant environmental substances (BMU 2008).

Long term storage is accomplished under conditions, which exclude condition change or loss of chemical characteristics, over a period of numerous decades. The archive stores samples for retrospective examination of such substances whose danger potential for the environment or for human health is today unknown.

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

2 Guideline Objective

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based and standardized methods, to avoid contamination and inhibit loss of chemical information. The exceptionally high demand of true quality results derives 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 WAGNER et al. (1993) version. It is conform to the VDI guideline 3792 sheet 11 (VDI 2007).so that the research results are comparable with other monitoring programs.

Transport, further sample treatment and storage as well as chemical analysis have to be done following the actual guidelines of the ESB.

3 Function of the Specimen Type

In contrast to evergreen conifers, the leaves of the summer green deciduous trees are only exposed during the vegetation period to environmental influences. 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 (ELLENBERG 1996). The natural distribution of the red beech is between Belarus and the Baltic to the Cantabrian Mountains of North Spain. The North-South distribution ranges from South England and Southern Sweden to the mountains of Sicily and the Balkans. The proportion of beech in Germany is approximately 14% of the total forest area of 10.8 million ha (BMVEL 2004).

The red beech is suitable for the ESB because of its importance as prime producer 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 air pollution impact and they act as a filter to flowing air.

The following criteria underline the suitability of the red beech for the ESB:

- availability of comprehensive baseline and comparative data e.g. through research programs and inventories of forest decline (e.g. KNABE 1982; BMELF 1990, 1995, 2000: FISCHER & LORENZ 2000: EC-UN/ECE 2000a, b; UN/ECE & EK 2000; BMVEL 2004),
- physiologically and eco-physiologically well studied species (e.g. DAVIDSON et al. 1992; MASAROVIČOVÁ et al. 1999; NEIRYNCK & ROSKAMS 1999; BAUMGARTEN et al. 2000; BORTIER et al. 2000; GÜNTHARDT-GEORG et al. 2000; SCHRAML & RENNEBERG 2000,
- usage of the beech as an indicator organism since the early Sixties (e.g. GUDERIAN & STRATMANN 1968; ZIMMERMANN 1986, 1989),

- continued leaf exposition, thus the leaves 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 evolvement leaves are coated with elementary hairs which rapidly dwindle through abrasion. Subsequently a homogeneous even leaf surface remains (NEINHUIS & BARTHLOTT 1998).

5 Predefinitions for the Sampling

5.1 Selection and Definition of Sampling Sites

Representative sampling sites of a sampling area, which normally covers several square kilometers, are selected using the stratified random sample principle after GREEN (1979).

Hence multiple (> 10) homogenous locations are chosen for a screening. Essential criteria for homogeneity are:

- distribution of the target tree species,
- age of the target tree species population,
- forest management,
- exposure.

The selected locations must contain a large number of suitable trees of various ages, to ensure long term sampling. On defining the sampling locations the minimum distance to major roads, railway lines, power lines, etc. should be at least 100m. For elevated traffic routes the distance should be at least a tenfold of the height of the interfering object above the sampling location.

From the selected location trees are sampled and tested for homogeneity by biometrical and analytical characterization. In case of adequate homogeneity the location is determined as a part of the sampling site.

Additionally, provision should be taken from the outset to ensure that the selected locations, under the aspect of forest management, forest protection and ownership are suitable for long term sampling sites This implies arrangements with the Forestry Authority and owners before starting. The sampling sites should be protected by contract and as far as possible, be excluded from usual forestry measures and other disturbing changes.

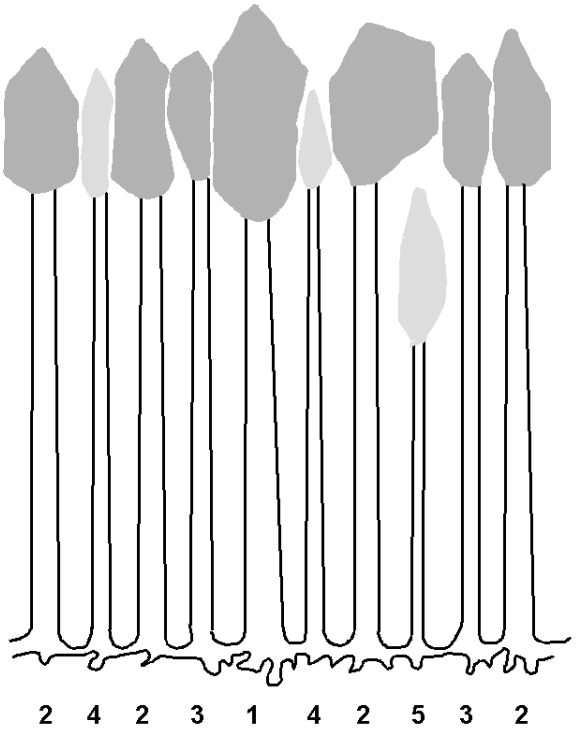

Fig. 1: Tree Categories by KRAFT (1884) (1 = pre-dominant, 2 = dominant, 3 = co-dominant, 4 = dominated, 5 = complete epigenous) (according to ASSMANN 1961)

5.2 Selection of Individuals and Sample Size

The sample size for the annual routine sampling is obtained by interpretation of the screening results.

For routine sampling of the ESB, samples should be taken from at least 15 trees per sampling site. By a given sample collective of 15 trees a minimum of 150 g fresh weight (leaves without stalks) per tree should be collected to represent the respective tree to a sufficient extent.

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 damages.

5.3 Sampling Period and Frequency

In long term programs as that of 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 which need to be substantiated in the area related sampling schemes. The sampling should be completed in the lower areas by the end of August and in higher by mid September.

With repeated sampling on the same site, sampling should be carried out depending on weather condition and phenology in the same determined period.

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. This includes amongst others:

- location and demarcation of the sampling sites,
- required sample size,
- time frame for sampling,
- addresses of the appropriate authorities
- useful addresses of tree climbers etc..

Describing the characteristic elements of the sampling sites within the area related sampling scheme secures long-term continuous sampling. In the case of changes within the sampling site or the sampled population the document has to be updated.

In case of major changes, so that comparability of the samples could not be guaranteed anymore, a new site has to be selected.

6 Implementation of the Sampling Procedure

All data collected in the course of sampling and through the biometric sample description are documented in the respective specimen data sheets (see appendix). A record is kept for each sampling with the following contents:

- all persons involved in the sampling,
- chronological procedure of the sampling,
- the underlying version of the sampling guideline and the area related sampling scheme for the current sampling,
- alterations to the sampling guideline and the area related sampling scheme.

Collecting specimens from the crown area of standing trees is performed by and exclusively permitted to 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 is used. This equipment must also fulfill actual safety requirements.

6.1 Required Equipment and Cleaning Procedures

Field work:

- specimen data sheets for documentation during the sampling
- several stainless steel scissors
- stainless steel trough to catch the cut leaves
- stainless steel containers (3.5 l or 5.5 l) with lids and fasteners
- waterproof pen for inscribing the paper bags and stainless steel containers
- stainless steel tweezers
- paper bags (1 bag per tree)
- disposable gloves
- scales (effective range up to at least 3 kg, reading accuracy 1 g)

- measuring tape for measuring the trunk circumference
- tree height measuring instrument
- air-thermometer (reading accuracy 1°C)
- soil-thermometer (reading accuracy 1°C)
- camera for documentation
- liquid nitrogen
- tools and protective clothing for liquid nitrogen handling
- cooling device (dewar vessel) 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 (80°C (+/–5°))
- precision scales (reading accuracy 1 mg)
- weighing pans
- stainless steel tweezers

Cleaning procedures:

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 (+/– 5°) for a minimum of an hour (sterilization). The containers remain closed 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 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.

At least four branches are taken from the crown of each tree. The branches should be evenly distributed in the free exposed upper crown as far as possible. Consideration is given that not too large branches are removed and that the bark is not damaged. Where there is sufficient space the branches are dropped. In the dropping procedure and where the branches hit the ground care must be taken that there is no contamination.

After biometric specimen description (chap. 7), the leaves without their stalks are cut using stainless steel cutters so that they fall directly into the container without being touched. Fig. 1 demonstrates the sampling procedure.

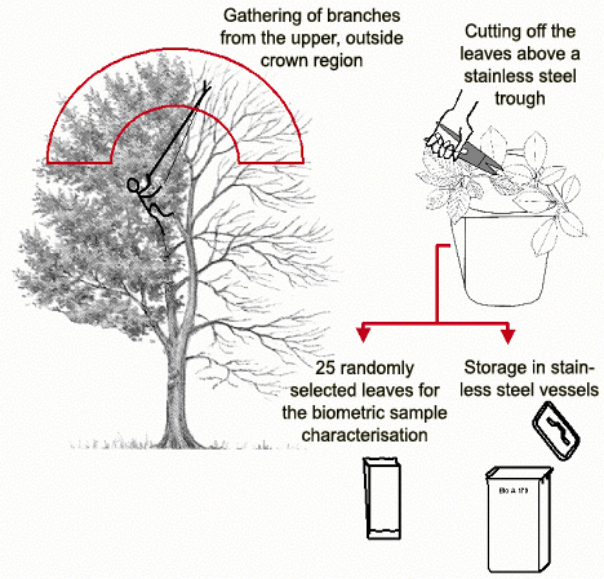

Fig. 2: Schematic representation of the sampling

For biometric sample characterization, 25 leaves are randomly taken out of the total sample amount using stainless steel tweezers and collected in a paper bag labeled with the respective tree number.

The remaining leaves are transferred from the stainless trough to the storage containers with gloved hands after it is has been weighed empty. After filling the container, the gross weight is determined and noted in the corresponding data sheet.

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 Characterization

On site the parameters for the sample description are recorded persistent with the respective

specimen data sheets (description of the tree and description of the leaves) prior to the removal of the leaves.

Using 25 randomly selected leaves per tree, the dry weight is determined (reading accuracy 0.01 g) in the laboratory. The paper bags containing the 25 randomly selected leaves per tree are placed in the drying cabinet (80°C (+/– 5°)) for drying (not too densely packed to prevent overheating), and left to dry for about 2 days (until weight stability is reached).

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Checklist to Prepare and Conduct the Sampling

Specimen Type:	**Red Beech (*Fagus sylvatica*)**
Target Compartments:	leaves without leaf stalks from at least 4 branches, upper, outer crown region
Individual Specimens:	predominant, dominant or co-dominant trees (tree category 1, 2 or 3 by KRAFT), older than 40 years
Random Sample Number:	at least 15 trees
Sample Quantity for the ESB	150 g fresh weight (= leaves without stalks) from 15 trees must be sampled to gain the needed quantity of 2.200 g
Sampling Period:	end of August until middle of September (prior to leaf discoloration)
Sampling Frequency:	1 sampling per annum
Equipment Required for Field Work:	• specimen data sheets for documentation during the sampling (sampling location, atmospheric condition, description of the tree and the leaves, storage) • several stainless steel cutters • stainless steel container (trough) to catch the cut leaves • waterproof pen to inscribe the paper bags and stainless steel containers • stainless steel tweezers, to select the leaves for the biometric characterization • disposable gloves • scales (effective range up to at least 3 kg, reading accuracy 1 g) • measuring tape for measuring the trunk circumference (reading accuracy 1 cm) • tree height measuring instrument • air-thermometer, soil-thermometer (reading accuracy 1°C) • camera for documentation
Sample Packing:	• stainless steel containers (vessels, 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 unit (dewar) for the 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 (80°C (+/– 5°)) • precision scales (reading accuracy 1 mg) • weighing pans • stainless steel tweezers
Biometric Sample Characterization:	tree (see specimen data sheets): • stand type, trunk diameter and tree height • fructification leaves: • damage (feeding on leaves, chlorosis, necrosis), contamination of 25 leaves: • dry weight of the leaves (reading accuracy 0.01 g)

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Specimen Data Sheet 1: Sampling Location

Red Beech (*Fagus sylvatica*)

Identification:

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

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

Tree Numbers: from __ __ to __ __

Gauß-Krüger-Coordinates:

Easting: __ __ __ __ __ __ __ Northing: __ __ __ __ __ __ __

Datum: _____ Ellipsoid: _____

Altitude: __ __ __ __ m (above sea level)

Slope Gradient: __ __ __ %

Exposure: _____

Size of the Sampling Location: __ __ km² __ __ ha __ __ a __ __ m²

Land Use: _____

Sampling Location: _____

Remarks: _____

_____

Person(s) in Charge:

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 in 1,5 m Height (°C)	__ __
__ __	Soil Temperature in 10 cm Depth (°C)	__ __
__ / 8	Cloud Covering	__ / 8
__	Type of Clouds	__
__ __ __	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 = Snow
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

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Specimen Data Sheet 3: Sample Description

Red Beech (*Fagus sylvatica*)

Identification:

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

Tree Number: __ __

Stand Type:

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

Trunk Circumference (in 1,3 m height): __ __ __ cm **Tree Height:** __ __ m

Location of Sampled Branches in Crown:

☐ Upper Outer Crown (Normal Case)
☐ Upper Inner Crown
☐ Lower Inner Crown
☐ Lower Outer Crown

Damages at Leaves

Feeding on Leaves:

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

Damage Type

☐ Nonexistent
☐ Leaf Skeletonizing
☐ Pitting
☐ Sucking Spots of Insects
☐ Mining
☐ 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

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Specimen Data Sheet 4: Specimen Description and Storage

Red Beech (*Fagus sylvatica*)

Identification:

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

Tree Number: __ __

Modification of or Overlay on Leaf Surface: (Estimation at 5% intervals)	**Type of Overlay**
Overall Top Side __ __ __ % Overall Bottom Side __ __ __ %	☐ Nonexistent ☐ Honeydew ☐ Sooty Mould ☐ Fungal Diseases on Leaf ☐ Gall Mite (Top Side) ☐ Gall Mite (Bottom Side) ☐ Gall Midges ☐ Other: _____

Fructification: ☐ Nonexistent ☐ Little ☐ Moderate ☐ Heavy

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]	Weighted Sample [g]	Remarks
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	
__ __ __ __ __ __ __	__ __ __ __	__ __ __ __	__ __ __ __	

Remarks: _____

_____

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Sampling Record

Red Beech (*Fagus sylvatica*)

Sampling Area: _____ Identification: __ __ __ __ __

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

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

1. Objective of the Sampling: _____

_____

2. Actual Timeframe of the Sampling:

Date	Time		Sample No.		Remarks
	from	to	from	to	

3. Participants: Conductor/Recorder: _____

Other: _____

_____

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

- ☐ 4.1 Sampling Period
- ☐ 4.2 Sampling Site and Sampling Location (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 of, and Reason for Possible Variations (Clear Text):

_____

_____

_____

_____

Remarks:: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature