

Guideline for Sampling and Sample Processing

Red Beech (*Fagus sylvatica*)

Julian Hans, Isabelle Junk, Roland Klein, Martin Paulus, Kathrin Tarricone, Diana Teubner

Trier University, FB VI – Biogeography
Universitätsring 15, 54296 Trier

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Appendices: Checklist to Prepare and Conduct the Sampling Specimen Data Sheets

**Guidelines for Sampling, Transport, Storage and Chemical Characterization of
Environmental and Human Samples
Status: February 2024, V 2.1.0**

1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an environmental monitoring instrument of the Federal Ministry for the Environment, Climate Action, Nature Conservation and Nuclear Safety (BMUKN) which is under the technical and administrative coordination of the Umweltbundesamt (UBA), Germany's environmental protection agency. The ESB collects ecologically representative environmental and human samples, stores and investigates them for environmentally relevant substances.

Specific operating procedures and the concept of the ESB are the basics for the ESB operations (Umweltbundesamt 2008, 2014, 2023).

The long-term storage is carried out under conditions which, as far 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 yet unknown. 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 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 the Tarricone *et al.* (2018) version.

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

The coordination of the sampling guideline with the VDI (The Association of German Engineers)

Standard 3957 page 11 (VDI 2019) 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 16% of the total forest area of 11.4 million ha (BMEL 2021).

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 (BMEL 2000, BMEL 2023, Fischer and Lorenz 2000, BMEVL 2004),
- physiologically and eco-physiologically well studied species (Neiryneck and Roskams 1999, Bortier *et al.* 2000, Günthardt-Georg *et al.* 2000, Tarricone *et al.* 2015, Gennaretti *et al.* 2020),
- usage of the beech as an indicator organism since the early 1960s (Guderian and Stratmann 1968, Zimmermann 1986, Amores and Santamaría 2003, Beş *et al.* 2019),

- continuous leaf exposure, thus the leaf pollutant content represents the total pollution per vegetation period of the sampling year.

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.

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) and
- free from intense biological (e.g. feeding on leaves) or mechanical damage.

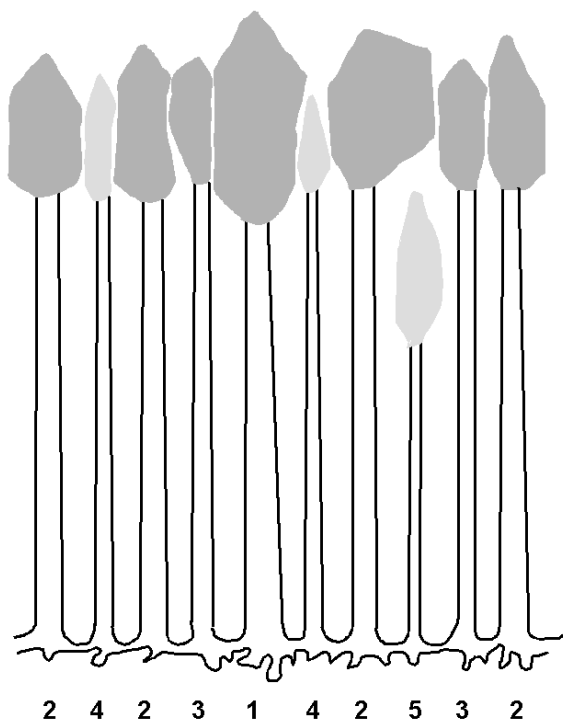


Abb. 1: Tree categories by Kraft (1884): 1 = pre-dominant, 2 = dominant, 3 = co-dominant, 4 = dominated, 5 = complete epigenous (from BMJ 2000)

5.3 Sampling Period and Frequency

For the ESB, sampling should be carried out either annually or bi-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,
- sample size,

- sampling period,
- appropriate authorities (e.g. forestry offices).

The objective of long-term and continuous sampling should be taken into account when drawing up and updating the area-related sampling schemes.

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. In special circumstances, e.g. a need to protect the trees to be sampled from damage caused by the climbing equipment, special equipment and climbing techniques that protect the trees are 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 with lids and clips,
- ziploc bag, DIN A4 or larger (1 bag per tree for 25 leaves),
- waterproof pen,
- weight for weighing down the biometric samples,
- powder-free disposal gloves,
- scale for checking the minimum sample quantity (weighing range up to at least 3 kg, reading 1 g),

- air thermometer,
- soil thermometer,
- cooling device for immediate deep-freezing and transport of the samples in the gas phase above liquid nitrogen (LIN),
- protective clothing for handling liquid nitrogen
- cooling device for cooling the biometric samples to 5°C ($\pm$ 3°C).

Laboratory:

- specimen data sheets,
- cooling device for cooling the biometric samples to 5°C ($\pm$ 3°C),
- leaf area meter,
- paper bags,
- cabinet dryer (80°C $\pm$ 5°C),
- scale (reading 0.01 g),
- weighing pans,
- powder-free disposal gloves.

Sample containers and all equipment are 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 a temperature of at least 120°C for a minimum of one hour (sterilization). Subsequently, the vessels cool in a closed drying oven. 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.

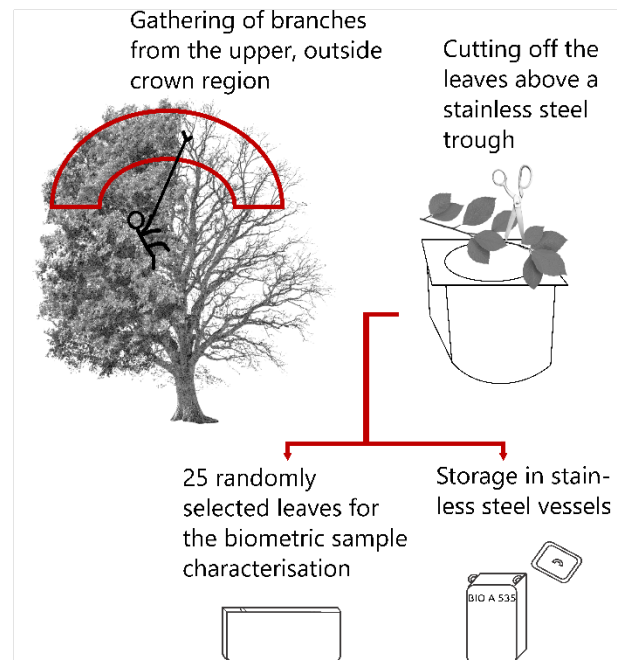


Fig. 2: Schematic representation of the sampling

After the sample characterization (Chap. 7), the leaves without their stalks are cut directly and without touching them into the stainless steel trough, using stainless steel scissors (Fig. 2). Laboratory gloves must be worn throughout the entire process and during the following steps.

To determine the leaf area and the dry leaf mass, a total of 25 randomly selected leaves are taken from each tree, collected in a ziploc bag labeled with the tree number, and stored at 5°C ($\pm$ 3°C) until the leaf area has been measured. The leaves should be spread out flat in the ziploc 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. 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 Sheets 3 and 4, except for leaf area and leaf dry weight, are to be recorded in the field at the branches obtained before the leaves are cut off.

In the laboratory, the 25 leaves collected per tree are measured with a leaf area meter. If possible, the measurement should be taken within one week and no later than two weeks after the sampling has been completed. The leaf area of the 25 leaves (reading to 1 cm²) is recorded in Specimen Data Sheet 4. The 25 leaves collected per tree are then transferred to paper bags labelled with the tree number and dried to constant weight (at least two days) in a cabinet dryer (at 80°C ± 5°C). Then, the dry weight is read (reading to 0.01 g).

The specific leaf area (SLA) and leaf mass per area (LMA) are calculated from the leaf area and leaf dry mass. LMA is the inverse of SLA. Both indices serve as a measure of the main function of the leaf, which is to absorb light for photosynthesis (Kadereit *et al.* 2014).

$$SLA = \frac{\text{leaf area [cm}^2\text{]}}{\text{leaf dry mass [g]}}$$

$$LMA = \frac{\text{leaf dry mass [g]}}{\text{leaf area [cm}^2\text{]}}$$

8 References

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Checklist to Prepare and Conduct the Sampling

Specimen Type	Red Beech (<i>Fagus sylvatica</i> 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 Kraft 1984), 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)
Required equipment for sampling	• specimen data sheets • stainless steel scissors • stainless steel container (trough) to catch the cut leaves • ziploc bag DIN A4 or larger (1 bag per tree) • waterproof pen to label the ziploc bag and stainless steel containers • powder-free disposable gloves • scale (weighing range up to at least 3 kg, reading 1 g) • air thermometer, soil thermometer • weight for weighing down the biometric samples
Sample packing	• stainless steel containers with lids and clips
Transport and interim storage	• cooling device for immediate deep-freezing and transport of the samples in the gas phase above liquid nitrogen (LIN) • cooling device for cooling the biometric samples to 5°C ($\pm$ 3°C)
Required equipment for laboratory work	• specimen data sheets • cabinet dryer (80°C $\pm$ 5°C) • paper bags • scale (reading 0.01g) • weighing pans • leaf area meter • powder-free disposal gloves
Sample characterization	• stand type, tree height • fructification • damage of leaves (feeding marks, chlorosis, necrosis), contamination • for 25 leaves: leaf area (reading 1 cm²) • subsequently on the same leaves dry weight (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:

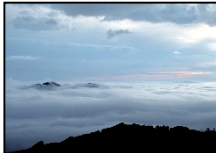
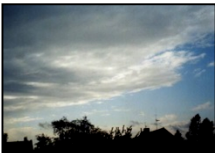
___ / X / ___ / ___ / ___

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 = 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

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 3: Description of the Tree

Red Beech (*Fagus sylvatica*)

Identification: ____ / **X** / ____ / ____ / ____

Tree number: ____

Stand Type:

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

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

Damage on 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

- | | |
|---------------------------------------|---|
| ☐ Non-existent | ☐ Leaf Skeletonizing |
| ☐ Pitting | ☐ Sucking Spots of Insects |
| ☐ Mining | ☐ Other: _____ |

Chlorosis

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

Chlorosis Type

- ☐ Non-existent
- ☐ As Stippling
- ☐ Blotchy, Skewbald

Chlorosis Dissemination on Leaf

- ☐ Non-existent
- ☐ In the Middle
- ☐ Tip Burn
- ☐ Marginal Scorch
- ☐ Interveinal
- ☐ On Whole Leaves

Necrosis

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

Necrosis Type

- ☐ Non-existent
- ☐ As Stippling
- ☐ Blotchy, Skewbald

Necrosis Dissemination on Leaf

- ☐ Non-existent
- ☐ In the Middle
- ☐ Tip Burn
- ☐ Marginal Scorch
- ☐ Interveinal
- ☐ On 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

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)

Type of Overlay

- ☐ Non-existent
- ☐ Honeydew
- ☐ Sooty Mould
- ☐ Fungal Diseases on Leaf
- ☐ Gall Mite (Top Side)
- ☐ Gall Mite (Bottom Side)
- ☐ Gall Midges
- ☐ Other: _____

Fruiting: ☐ none ☐ medium ☐ strong

Leaf Area of 25 Randomly Selected Leaves: ____ cm²

(or sum of partial measurement 1 = ____ , ____ cm² & partial measurement 2 = ____ , ____ cm²)

Date: _____ Signature: _____

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

Date: _____ Signature: _____

Storage

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

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

Remarks: _____

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. Objective of the Sampling: _____

2. Actual Timeframe of the Sampling:

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

3. Participants internal _____

external _____

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

- | | |
|--|---|
| ☐ 4.1 Sampling period | ☐ 4.6 Sampling technique / method of capture |
| ☐ 4.2 Sampling site and sampling point (selection/definition) | ☐ 4.7 Sampling amount |
| ☐ 4.3 Selection of the individual specimens | ☐ 4.8 Data collection |
| ☐ 4.4 Technical preparations | ☐ 4.9. Transport and interim storage |
| ☐ 4.5 Cleaning procedures for the packages | |

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

Remarks: _____

Recorder _____ Date _____ Signature _____