UMWELTPROBENBANK DES BUNDES

Guideline for Sampling and Sample Processing

Lombardy Poplar (*Populus nigra* 'Italica')

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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 (Commission on Air Pollution Prevention VDI and DIN), 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.

Due to its frequent and regular occurrence in densely populated and agricultural areas, the lombardy Poplar (*Populus nigra* 'Italica') is suitable as a indicator species for urban areas. It is a male clone of the European black poplar (*P. nigra* L.), which was created by a unique mutation and is vegetatively propagated as a clone and spread anthropogenically.

The following criteria underline the appropriateness of the use of the Lombardy Poplar for the ESB:

- it is found in almost all industrialized countries as an ornamental tree and for windbreak planting in residential and agricultural areas (FAO 1979),
- great genetic analogy (clone),
- great ecological valence and high resistance to environmental influences (i.a. Dimitri 1973),
- physiologically and eco-physiologically highly investigated species (i.a. Joachim 1953; Sebald 1959; Cannon *et al*. 1972; Severin and Köster 1982, Ksiazek *et al.* 1984, Omasa *et al.* 2000),
- continuous leaf exposure: with only one sampling event per annum, the pollution burden of the leaves represents the integrated effective dose of the main vegetation period,
- existence of comprehensive knowledge as an accumulation indicator in field tests (i.a. Hallez *et al*. 1979; Claussen and Bartels 1982, Dittmann *et al.* 1984, Grimmer *et al*. 1985, Wagner 1987, Capelli *et al.* 1989, Terhorst and Wittig 1988/89, Djingova *et al*. 1993, 1995,

1996, 1999, 2001, Sawidis *et al.* 1995, Marth *et al.* 1999).

4 Target Compartments

In accordance with the ESB, leaves without stalks are collected as target compartments. The dense foliage and the very prominent reticular venation are the reason why leaves of the poplar are highly effective in accumulating and binding a considerable quantity of particles (Sawidis *et al*. 1995).

As the accumulation of pollutants is related to the length of exposure, the leaves' age plays a crucial part in the evaluation of environmental samples. Due to the continuous growth of the poplar throughout the entire summer, the youngest leaves atop the long shoots must not be sampled.

5 Predefinitions for Sampling

5.1 Selection and Definition of Sampling Sites

To determine the sampling site(s) and the sample size, a screening should be performed before the first sampling. The aim of this preliminary investigation is

- the determination of the availability and spatial distribution of the sample type,
- the range of characteristics and material composition of the spatial pattern of pollution.

The first step in the screening is to map all sites covered with Lombardy Poplars that are suitable for sampling. For this, the criteria listed in chapter 5.2 are to be considered.

On the preselected sites, samples of at least 30 individuals are taken and analyzed individually. At least 3 (better 6) trees should be selected from each site.

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 of 15 trees, at least 75 g fresh weight (= leaves without stalks) should be collected to sufficiently represent each tree and reach the required total sample quantity of 1,100 g.

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

- 20 years old or older, to exclude the juvenile stage,
- with branches from the base on upwards,
- free from intense biological (e.g. crown dieback) or mechanical damage.

Specimens with early yellowing leaves, rust infestation (over 10% of the leaves), serious aphid infestation and those with chlorosis, necrosis, or damage through feeding on leaves are not selected unless this feature characterizes the average condition at the sampling location.

The trees selected for sampling must be free-standing without being considerably shielded by buildings, vegetation etc. and not located near local sources of emission.

5.3 Sampling Period and Frequency

For the ESB, a 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 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. parks department).

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.

6.1 Required Equipment and Cleaning Procedures

Field work:

- specimen data sheets,
- shears with telescopic handles extendable to a length of 5 m,
- 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 are cleaned in a laboratory washer using a chlorine-free powerful washing agent in a first step. After cold and hot (ca. 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 under 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.

Samples are taken at a height of 5 to 7 m above ground and distributed evenly over the different exposure directions.

The branches are cut off using a pair of shears with telescopic handles, extendable to about 5 m. From each tree at least 4 branches are taken from the periphery of the crown (see Fig. 1). In the dropping procedure and where the branches hit the ground care must be taken that there is no contamination. The cut branches are placed in the shade until they are processed.

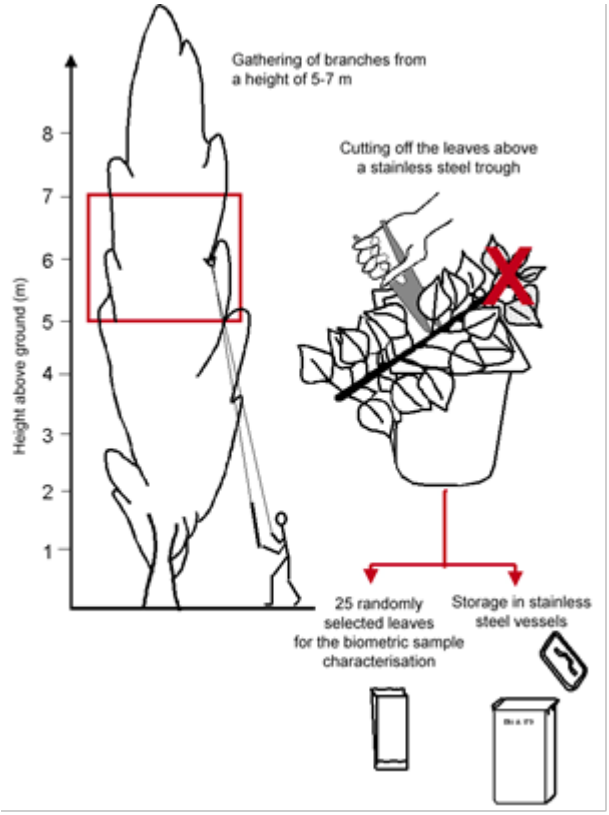

Fig. 1: Schematic representation of the sampling

After the sample characterization (chap. 7), the leaves without their stalks are cut directly and without any additional contact into the stainless steel trough, using stainless steel scissors. The youngest leaves at the top of the long shoots are not used (Fig. 1).

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 for the branches obtained before the leaves are cut off.

To determine the dry weight (reading 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	**Lombardy Poplar (*Populus nigra* 'Italica')**
Target Compartments	leaves without leaf stalks
Individual Specimens	poplars > 20 years (to exclude the juvenile stage)
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	Early August until middle of September (prior to leaf discoloration)
Sampling Frequency	1 sampling per annum
Required Equipment 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 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 (approx. 80°C) • scale (reading 0.01 g) • weighing pans • stainless steel tweezers
Sample Characterization	• stand type, tree height • leaf damage (eating damage, chlorosis, necrosis), contamination • for 25 leaves: leaf dry weight (reading 0.01 g)

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

Lombardy poplar (*Populus nigra* ‚Italica')

Identification:

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

- Specimen Type
- Specimen Condition
- Collection Date (MM/YY)
- 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

Lombardy poplar (*Populus nigra 'Italica'*)

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

Tree Numbers: from __ __ to __ __

Last precipitation date preceding the sampling: __ __ . __ __ . __ __

Type of precipitation: ____ (see table below)

Start of Sampling		**End of 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 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, move medium sized branches
6 = strong wind, moves thick branches
7 = stiff wind, shakes trees

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Specimen Data Sheet 3: Description of the Tree
Lombardy poplar (*Populus nigra* ‚Italica')

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

Tree Number: __ __

Stand Type:
- ☐ Grove
- ☐ Tree Row
- ☐ Free Standing Solitary Trees
- ☐ Marginal Stand Zone
- ☐ Forest Aisle

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

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

Leaf Damage (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
- ☐ On Leaf Edge
- ☐ In Interveinal Leaf Zones
- ☐ At Whole Leaves

Necrosis

__ __ __ %

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

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

Necrosis Dissemination on Leaf
- ☐ Nonexistent
- ☐ In the Middle
- ☐ Tip Burn
- ☐ On Leaf Edge
- ☐ In Interveinal Leaf Zones
- ☐ At Whole Leaves

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Specimen Data Sheet 4: Sample Description and Storage
Lombardy poplar (*Populus nigra* ‚Italica')

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 (light bluish dots, or confluent) ☐ Sooty Mold ☐ Rust Fungus Infection ☐ Other Fungal Diseases on Leaves ☐ Galls on Leaves ☐ Other: _____ _____

Petiole Gall Infestation __ __ __ % of the leaves (estimate in 10% steps)

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

Storage

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

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

Remarks: _____
_____

Dry weight determined (date): Signature:

GERMAN ENVIRONMENTAL SPECIMEN BANK

Sampling Protocol

Lombardy poplar (*Populus nigra* ‚Italica')

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	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