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Guidelines for Chemical Analysis

Determination of the Elemental Content of Environment Samples using ICP-OES

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Umwelt
Bundes
Amt
Für Mensch und Umwelt

Contents

Guidelines for sampling, transport, storage and chemical characterisation
of environmental and human samples
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1 German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument for the monitoring of the environment. It is in the responsibility of the Federal Ministry for the Environment, Nature Protection and Reactor Safety (BMU) and technically and administratively coordinated by the Federal Environment Agency (Umweltbundesamt). The ESB collects ecologically representative environmental specimens as well as human samples, stores them and examines the archived material for environmental relevant substances.

The long-term storage is performed under conditions that exclude a change of state or a loss of chemical characteristics as far as possible during a period of several decades. By this means the archive provides specimens for a retrospective monitoring of such substances, whose hazard potential for the environment or human health are not yet known.

Comprehensive information on the German ESB is available at www.umweltprobenbank.de (English language pages available).

2 General Information

This guideline for the 'Determination of the Elemental Content of Environment Samples Using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)' describes a method for quantifying the dissolved elements barium, calcium, cadmium, iron, potassium, magnesium, manganese, phosphorus, sulphur and zinc in digests of plant and animal sample material. The can also be used for determining the content of other elements detectable by means of ICP-OES in biological and environmental samples.

This guideline is a continuation of the method guidelines entitled 'Analytic Regulations for Elements and Species in Environmental Samples, Matrix Group 1', developed by the Environmental Specimen Bank for analysing digests of environmental samples (UMWELTBUNDESAMT 1996). It includes references from the German standard DIN EN ISO 11885 / DEV E22 'Water Quality – Determination of 33 Elements by Inductively Coupled Plasma Atomic-Emission Spectrometry (1998)' which is used for analysing acidic aqueous solutions (e.g. acidified drinking water, waste water and other water samples, *aqua regia* extracts from soils and sediments).

3 Field of Application

This guideline describes the procedure for examining digestions of environmental samples by means of ICP-OES.

In the program of analysis pursued by the Environmental Specimen Bank, this guideline is regularly used for samples of the following types: bladder wrack (brown algae), spruce shoots, pine shoots, poplar leaves, beech leaves, earthworm. The method described below can also be applied to other biological sample types. Where samples are used for which no empirical data are available, a suitable validation process should be applied (see section 8.6).

In each case, the working range of the method has to be adapted to the concentration of the elements in the digestion solutions. The lower range of application of the process described depends on the matrix in use and the errors connected therewith. For digested biological samples the typical limits of detection normally achieved are listed in table 1 (data refer to dry weights). In some cases, lower limits of detection are achievable, but the content of elements in the samples concerned here are higher.

The wavelengths are selected in accordance with the required limit of detection (section 8.3) and the possibility of interference by other elements present in the digestion solution (section 9).

Table 1: Recommended wavelengths to DIN EN ISO 11885 and ESB guidelines 'Analytic Regulations for Elements and Species in Environmental Samples, Matrix Group 1' and typical limits of detection normally achieved by the method in the selected working range for the wavelengths used for environmental samples (**bold**).

Element	Wavelengths [nm]	Limit of detection [µg/g]#
Barium (Ba)	233.527	-
	455.403	0.3 – 0.6
	493.409	-
Calcium (Ca)	315.887	-
	317.933	350
	393.366	
	422.673	-
Iron (Fe)	**259.940**	0.9 – 1.2
	238.20	-
Potassium (K)	**766.490**	100
	769.90	-
Magnesium (Mg)	**279.079 $**	1.0
	279.553	-
	285.213 *	40
Manganese (Mn)	**257.610 §**	2.0
	293.306	-
	293.931 $	5.0
Phosphorus (P)	**178.287**	170
	213.618	-
	214.914	-
	177.428	-
Sulphur (S)	**182.036**	2.0 – 100
	180.669	-
Zinc (Zn)	206.191	-
	213.856	0.5 – 0.6

Calculation of solid content on the assumption that 200 mg of solid material is used for the digestion and then replenished to 20.0 mL.

§ Wavelength of this element used for plant samples.

* Wavelength of this element used for earthworm samples.

$ Wavelength of this element used for bladder wrack samples (source: Thermo Instruments).

4 Terminology

4.1 Sensitivity

Slope of the regression lines indicating the relationship of the intensity of the signal to the concentration of an element.

4.2 Reagent blank value

An aqueous solution which has the same acid concentration as the calibration solutions and is treated in the same way as the digestion solutions under analysis. The purpose of reagent blank values is to identify the possible contamination by reagents, digestion and digestion vessels.

4.3 Blank value

An aqueous solution which has the same acid concentration as the calibration solutions. The purpose of blank values is to check whether sample material has been carried-over from previous measurements and to ensure that the set rinsing time between measurements is adequate.

4.4 Interference

Interference is defined as any effect which distorts the signal where the concentration of the element being tested remains constant. A distinction is made between spectral interference (undesirable signals which interfere with the signal from an element), chemical interference (chemical reactions which reduce the concentration of an element) and physical interference (e.g. caused by differences in viscosity between standard and sample solutions).

5 Description of the method

Elements in solution can be detected and quantified by means of optical emission spectrometry with inductively coupled plasma (ICP-OES; also ICP-AES, ICP atomic emission spectrometry). The solution under analysis is nebulised and the aerosol thus formed transported to a high-frequency plasma in which the constituents of the solution are atomised and partially ionised. The characteristic emission lines of the atoms and ions are dispersed by a monochromator or polychromator and the intensity of the lines recorded with a suitable detector. The measurement can be done axially or radially. The axial measurement is more sensitive. Devices are available which permit both types of measurement.

The quantities are determined by means of calibration with suitable standard solutions because there is a linear relation between the signal intensities of the emission and the concentration of an element.

6 Apparatus

6.1 Vessels for element solutions

The stability of diluted element solutions (both sample and standard solutions) is determined substantially by the material of the vessels used. The suitability of the material for the intended purpose must always be ensured beforehand. For determining elements in the trace range, vessels of glass or polyvinyl chloride (PVC) should not be used. Vessels made of perfluoralkoxy plastics (PFA), hexafluoro-ethylene-propylene (FEP) or quartz are more suitable. In many cases, high-density polyethylene (e.g. HDPE vessels which are used for scintillation measurements) and polypropylene may also be used. When they are being re-used, the vessels must be rinsed with nitric acid or ‘steam cleaned’ with boiling concentrated nitric acid in closed systems.

6.2 Emission spectrometer

Optical emission spectrometer with inductively coupled plasma consisting of:

- computer-controlled emission spectrometer with background correction;

- radio-frequency generator;

- Argon gas supply (minimum purity 99.99 %).

7 Reagents

7.1 General

All chemicals used must be suitable for trace analysis. The digestion reagents must be of at least high-purity quality. The reagents should be selected to ensure that the contents of the elements to be determined or of possibly interfering elements should be negligible in relation to the lowest element concentration to be determined.

NOTE: For element analysis carried out by the Environmental Specimen Bank, acid produced in-house by sub-boiling is generally used. Every charge is analysed to determine the content of the elements being analysed. The concentration of the sub-boiling nitric acid is determined via the density (> 1.39 g/mL at room temperature equals $\geq$ 65% by weight).

7.2 List of reagents

7.2.1 Water from a high-purity water supply system. Quality: specific resistance > 18.2 MΩ cm.

7.2.2 Nitric acid, $\rho(HNO_3) > 1.39$ g/ml ($\geq$ 65 %).

7.3 Element stock solutions

Commercially available solutions of the elements Ba, Ca, Fe, K, Mg, Mn, P, S, Zn (e.g. 1000 mg/L each), as single or multi-element solutions. The solutions are usable for several years. The shelf life stated by the manufacturer should always be observed. Only standard solutions with certificate should be used.

7.4 Multi-element standard solutions

The concentration of the elements in the standard solutions should be selected to ensure that the working range is covered. If necessary, further elements should be added to adapt the reference solution to the sample matrix.

The shelf life of diluted element solutions (1 to 100 µg/L) is limited. These should be replaced at regular intervals, and at the latest, every three months. Where solutions with low concentrations are required, these should be mixed freshly each day.

NOTE: For the analysis of samples for the Environmental Specimen Bank, multi-element standard solutions are used which are either purchased externally or prepared from single-element standards of the elements Ba, Ca, Fe, K, Mg, Mn, P, S and Zn. To prepare the actual standard solutions, the solutions are diluted before the measurement on the day they are required.

8 ICP-OES measurement procedure

8.1 General remarks

Optical emission spectrometers with inductively coupled plasma (ICP-OES) have a linear relation several times the power of ten between the intensities measured and the concentration of an element. This means that quantification by means of external calibration with a linear regression line is possible. The calibration should be adjusted to the working range in question. Even for the lowest concentration values, sufficient measuring sensitivity and reproducibility must be assured.

8.2 Preparation of the apparatus

The ICP-OES should be started and set in accordance with the instructions of the manufacturer. To ensure sufficient stability of the plasma, it should be started up at least half an hour before the measuring operation.

The wavelengths for the element determinations are set according to table 1.

8.3 Calibration

Calibration is carried out on a daily basis using the appropriate program options of the apparatus. To do this, multi-element standard solutions (see section 7.4) are diluted according to the required working range. The type and concentration of acid in standard and sample solutions must be adapted to suit one another.

If the contents of the individual elements in the samples differ widely, it may be necessary to use calibration solutions of different concentrations for these elements.

For each element being analysed, one straight calibration line with at least four concentrations and a blank-value solution should be determined (multi-element standard solutions to 7.4). The highest standard concentration for a calibration should be greater than the lowest concentration by a factor of 10-20 at most. If the working range is greater, more calibration solutions should be prepared. Each value measured is an average of at least three measurements carried out automatically by the unit.

The linearity within the working range should be verified. The coefficient of correlation r should be > 0.995. Should r < 0.995 and the calibration still be used, the reason must be explicitly stated (e.g. calibration in the lowest application range of the method and consequent decrease in measurement accuracy).

For each measurement, the limit of quantification of the method must be stated. This may be calculated using the blank-value method (estimation of the limit of detection according to the German standard DIN 32645, 1994; the limit of quantification is calculated as three times the respective limit of detection).

8.4 Check on equipment function

For each series of samples, suitable standard solutions containing the elements in question should be measured to check the function of the unit. These test measurements are carried out in order to detect any inaccuracies or other problems in the unit. Should the established limits be exceeded, the measurement must be repeated.

NOTE: When analysing samples for the Environmental Specimen Bank certified aqueous reference materials are also analysed to detect any errors in the system. For concentrations of more than five times the limit of quantification, 85-115% of the certified value should be achieved, and for concentrations between the limit of quantification and five times the limit of quantification, this should be 80-120%.

8.5 Measurement of digestion solutions

Following calibration of the emission spectrometer and verification of its correct function, the digestion solutions are to be analysed. The samples are analysed under the same conditions as the standards (in particular adjusted acid concentration).

If necessary, samples are diluted in order to be analysed within the working range of the method.

Table 2: Selection of certified reference materials (solids).

Name	Code	Certified by	Certified for
Beech leaves	CRM 100	BCR	Ca, K, Mg, P, S
Spruce needles	CRM 101	BCR	Ca, Fe, Mg, Mn, P, S, Zn
Poplar leaves	NCS DC 73350	Institute of Geophysical and Geochemical Exploration (Langfang, China)	Ba, Fe, Mg, Mn, Zn
Pine needles	NIST 1575a	NIST	Ba, Fe, Mg, Zn
Bovine liver #	NIST 1577b	NIST	Ca, K, Mg, P, S
Sea lettuce (algae)	CRM 279	BCR	Zn

as reference material for matrix earthworm.

The dilution must be taken into account in calculating and recording the result.

The correctness of the calibration is verified regularly. After about 20 samples, a digested certified reference material with as similar a matrix as possible and similar concentration of elements is analysed in the same way as the samples.

In addition to the sample solutions, blank solutions also continue to be analysed at regular intervals to avoid potential carry-over.

Should the specified quality requirements not be met by one series of analyses, these should be repeated after renewed calibration of the unit.

NOTE: When analysing samples for the Environmental Specimen Bank, certified reference materials are also analysed (for examples, see table 2) to detect any problems caused by incomplete digestion or losses. With concentrations of more than five times the limit of quantification, 80-120% of the certified values should be achieved, and for concentrations between the limit of quantification and five times the limit of quantification, this should be 75-125%. When analysing material for the Environmental Specimen Bank, ESB reference materials (ESB-RM) are also analysed, i.e. similar samples from earlier years, for which analysis results exist in the information system of the ESB (IS UPB). The following quality criteria apply for such samples: for concentrations of more than five times the limit of quantification, 75-125% of the certified values should be achieved, and for concentrations between the limit of quantification and five times the limit of quantification, this figure should be 70-130%.

8.6 Validation of method

For method validation, standard addition tests can be carried out. Different concentrations of the element being determined (e.g. approx. 50% and 100% of the concentration anticipated in the sample) are added to aliquots of that sample. These partial samples along with a further partial sample are then analysed. By extrapolating the straight lines (measurement signal as linear function of the mass concentration of the analysed element) it is now possible to determine the original concentration of the sample. This procedure is described in detail in the German standard DIN 32633 (1998).

Another important means of method validation is the analysis of certified reference materials which are similar to the matrix. The analysis of replicates of such samples provides data on correctness, reproducibility and accuracy.

With concentrations substantially above the limit of quantification, it is also possible to use the dilution method to test the analysis process. To do this, a sample is first analysed in the undiluted state and then after dilution by a factor of 5 to 10. If no interference occurs, the results from both analyses should be identical with due consideration of dilution and measurement uncertainty. The criterion of quality is 100 ± 10 %.

For method validation, at least the following process parameters should be determined:

Selectivity / specificity: these are met if the figure measured for the chemical blank value is less than the lowest validated concentration.

Reproducibility: the reproducibility is calculated from the correspondence data of the reference materials via the relative standard deviation (S_{rel}). This condition is fulfilled if the following applies: $S_{rel} < 10$ % (where $n \geq 5$).

Lowest limit of measuring range: the lowest limit of the method is the calibration point for which the signal-noise ratio is no less than 6:1.

Limit of detection / limit of quantification: see section 8.3.

For the complete validation of a process, it is advisable to carry out reference analyses using different methods (e.g. ICP mass spectrometry).

9 Interferences

Interferences can be divided into various categories (for details see DIN ISO 11885).

Spectral interferences (for those relevant, see table 3):

- line coincidence (can be compensated by means of algorithms);
- band coincidence (can be compensated by using different wavelengths).

Background disturbance (can be compensated by a background correction in the vicinity of the line being analysed):

- by continuous or recombination phenomena;
- by stray light from one band emission to another (more highly concentrated) element.

Chemical and physical interferences (to be avoided by adjusting the composition of sample and calibration solutions or by using standard addition for quantification):

- caused by the formation of molecules or by ionisation and evaporation effects;
- effects linked with the transport of the samples to the nebuliser and the nebulising process itself (e.g. altered viscosity or surface tension depending on different acid concentration and particle content).

Table 3: Possible spectral interferences according to DIN EN ISO 11885 for different wavelengths (**bold**: for lines used in analysis of ESB samples).

Element	Wave-lengths [nm]	Possible spectral interference by
Barium (Ba)	**233.527**	Fe, V
	455.403	-
	493.409	-
Calcium (Ca)	315.887	Co
	317.933	Fe, V
	393.366	-
	422.673	No data
Iron (Fe)	**259.940**	-
	238.20	Co
Potassium (K)	766.490	Mg, Ar
	769.90	-
Magnesium (Mg)	**279.079**	-
	279.553	-
	285.213	Fe
Manganese (Mn)	**257.610**	Fe, Mo, Cr
	293.306	Al, Fe
	293.931	Fe
Phosphorus (P)	**178.287**	I
	213.618	Cu, Fe, Mo, Zn
	214.914	Cu, Al, Mg
	177.428	Cu
Sulphur (S)	**182.036**	Cr, Mo
	180.669	Ca
Zinc (Zn)	206.191	Cr
	213.856	Cu, Ni, Fe

For elements whose determination may be affected by interference, or which have a tendency to carry-over due to the matrix analysed, the lower application range may be above the values listed in table 1.

10 Evaluation

The analysis data are evaluated by the software in the emission spectrometer. The automatic evaluation must be verified for plausibility. During evaluation, the analysis data are calculated in accordance with any corrections which may have been selected (see section 9). The software in the unit calculates the linear regression of the linear regression lines in order to calculate the slope and ordinate intercepts as well as the coefficients of coordination (r). The concentrations of all the analysis solutions (blank values, aqueous reference materials, digestion solutions) are determined on the basis of the straight calibration lines.

Should the concentration of an element in the blank-value sample be higher than 10% with reference to the concentration in the sample, the values are adjusted by the blank value (i.e. the concentration measured as blank value is subtracted from this measurement value).

Any dilution operations must also be taken into account in all calculations. It must be possible to reconstruct all the calculations carried out by means of the electronically stored data and documented by means of printouts.

When examining digested samples of solids, the results must refer to the solid (i.e. to dry mass or fresh mass). The concentrations of the elements in the digestion solutions are generally determined by the ICP-OES instrument software. The further calculation of the element contents in the solid matter is done by means of the following equation:

$$\omega_E = V / M * \rho_E * F$$

where:

ω_E	proportion by mass of the element in the solid matter, stated, for example as µg/g;
M	mass of the sample used, stated in mg (e.g. 200 mg);
V	volume to which replenished, stated in mL (e.g. 20,0 mL);
ρ_E	concentration of the element under consideration in the digestion solution, stated for example in µg/L;
F	conversion factor (1 L/mL * mg/g).

If the relative standard deviation of the average value of the results of several measurements (from n = 6) is > 3 %, outlier tests are carried out according to Nalimov and Shapiro-Wilk. A maximum of two values are eliminated as outlier.

11 Statement of results

The results refer to the amount of solid material used (dry mass or fresh mass).

All results should be stated to three significant digits.

EXAMPLES: Manganese (Mn) 1230 µg/g; Calcium (Ca) 123 µg/g; Zinc (Zn) 12.3 µg/g; Magnesium (Mg) 1.23 mg/g; Sulphur (S) 0.123 mg/g.

Measurement results are subject to a degree of uncertainty. The measurement uncertainty is a parameter assigned to the result and indicates the dispersion of those values which can be reasonably associated with the measured variable. In the working range of a process, the measurement uncertainty increases as the concentration in the sample decreases (i.e. the lower the concentration the greater the uncertainty). The degree of uncertainty of a measured figure can be determined in a number of ways which are described in 'ISO Guide to the Expression of Uncertainty in Measurement (GUM)' (ISO, 1995) and guideline 'Quantifying Uncertainty in Analytical Measurement' (EURACHEM/CITAC, 2000). A practical means of determining uncertainty is the so-called Nordtest process (MAGNUSSON ET AL., 2003; calculation from duplicate measurements of certified reference materials and ring-test results).

NOTE: For the analysis of Environmental Specimen Bank samples, generally six sub-samples from one homogenate are used. The standard deviation of the average value is regarded as the measurement uncertainty of the result. The correctness of the results is verified with the help of certified reference materials. Representative data are given in the appendix.

12 Analysis report

The following data should be documented in the analysis report:

- Reference to this guideline,
- Unique sample designation,
- Data on preliminary sample treatment and digestion,
- Concentration of the elements in question with reference to the percentage of solid matter (depending on material used, with reference to fresh or dry mass),
- Dry weight (if applicable),
- Statement of measurement uncertainty if applicable,
- Any observations or comments,
- Any deviations from this guideline.

13 Representative analysis results

Representative results of analyses are given in the appendix:

a) Results of the analysis of certified reference materials,

b) Results of the analysis of reference materials from the Environmental Specimen Bank,

c) Results of the analysis of representative samples from the Environmental Specimen Bank.

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Appendix: Representative Analysis Results

a) Results of the analysis of certified reference materials.

Reference material	Element	Certified content	Correspondence	Comment
Pine needles NIST 1575a	Ba	6.00 µg/g	88.6 ± 0.8 (n = 2)	Certified value
	Fe	46.0 µg/g	99.8 ± 6.8 (n = 2)	Certified value
	Mg	1.06 mg/g	86.8 ± 1.0 (n = 2)	Certified value
	Mn	488 µg/g	92.4 ± 0.5 (n = 2)	Certified value
	Zn	38.0 µg/g	96.3 ± 1.7 (n = 2)	Certified value
Spruce needles BCR CRM 101	Ba	9.49 µg/g	108.1 ± 2.3 (n = 5)	Own average value
	Fe	151 µg/g	99.7 ± 3.1 (n = 5)	Certified value
	Mg	0.619 mg/g	92.8 ± 2.9 (n = 5)	Certified value
	Mn	915 µg/g	99.4 ± 1.0 (n = 5)	Certified value
	S	1.70 mg/g	92.8 ± 1.2 (n = 5)	Certified value
	Zn	35.3 µg/g	89.7 ± 1.9 (n = 5)	Certified value
Beech leaves BCR CRM 100	Fe	553 µg/g	95.2 ± 1.7 (n = 8)	Certified value
	Mg	0.878 mg/g	96.4 ± 1.4 (n = 8)	Certified value
	Mn	1300 µg/g	98.5 ± 1.6 (n = 8)	Certified value
	S	2.69 mg/g	95.1 ± 1.1 (n = 8)	Certified value
	Zn	65.7 µg/g	85.8 ± 2.2 (n = 8)	Certified value
Poplar leaves NCS DC73350	Ba	26.0 µg/g	97.4 ± 1.8 (n = 4)	Certified value
	Fe	274 µg/g	86.6 ± 0.9 (n = 4)	Certified value
	Mg	6.50 mg/g	92.8 ± 1.0 (n = 4)	Certified value
	Mn	45.0 µg/g	103.1 ± 0.8 (n = 4)	Certified value
	Zn	37.0 µg/g	96.7 ± 2.2 (n = 4)	Certified value
Sea lettuce BCR CRM 279	Fe	2300 µg/g	96.7 ± 2.7 (n = 5)	Information value
	Mg	14.4 mg/g	95.4 ± 1.4 (n = 5)	Information value
	Mn	2030 µg/g	91.6 ± 1.2 (n = 5)	Information value
	Zn	51.3 µg/g	89.9 ± 5.9 (n = 5)	Certified value
Bovine liver NIST 1577b	Ca	0.116 mg/g	107 ± 2 (n = 6)	Certified value
	K	9.94 mg/g	92.0 ± 0.3 (n = 6)	Certified value
	Mg	0.601 mg/g	90.6 ± 0.3 (n = 6)	Certified value
	P	11.0 mg/g	86.2 ± 1.0 (n = 6)	Certified value
	S	7.85 mg/g	88.4 ± 0.8 (n = 6)	Certified value

b) Results of the analysis of reference materials from the Environmental Specimen Bank (IS UPB – Information system of the Environmental Specimen Bank).

Reference material	Element	Concentration (aus IS-UPB)	Correspondence in %
Pine shoots Dübener Heide 1992	Ba	4.12 µg/g	91.0 ± 0.2 (n = 2)
	Fe	277 µg/g	89.7 ± 1.5 (n = 2)
	Mg	0.829 mg/g	83.3 ± 7.2 (n = 2)
	Mn	301 µg/g	90.9 ± 0.9 (n = 2)
	S	1.58 mg/g	88.1 ± 0.6 (n = 2)
	Zn	41.0 µg/g	97.0 ± 5.8 (n = 2)
Spruce shoots Berchtesgaden 1985	Mg	1.23 mg/g	86.1 ± 2.8 (n = 4)
	Zn	95.7 µg/g	95.7 ± 4.4 (n = 4)
Beech leaves Hochharz 1996	Ba	16.4 µg/g	88.0 ± 0.5 (n = 7)
	Fe	81.0 µg/g	95.0 ± 1.3 (n = 7)
	Mg	1.08 mg/g	85.8 ± 0.8 (n = 7)
	Mn	794 µg/g	91.4 ± 1.1 (n = 7)

Reference material	Element	Concentration (aus IS-UPB)	Correspondence in %
	S	1.58 mg/g	84.7 ± 0.6 (n = 7)
	Zn	33.6 µg/g	79.3 ± 2.1 (n = 7)
Bladder wrack Eckwarderhörne 1989	Ba	33.7 µg/g	85.4 ± 1.3 (n = 4)
	Fe	489 µg/g	88.2 ± 0.6 (n = 4)
	Mg	8.54 mg/g	88.9 ± 0.7 (n = 4)
	Mn	298 µg/g	91.3 ± 2.5 (n = 4)
	S	21.4 mg/g	100.5 ± 2.0 (n = 4)
	Zn	148 µg/g	81.7 ± 1.3 (n = 4)
Earthworm Saartal 1996	Ca	7.56 mg/g	89.1 ± 2.3 (n = 6)
	K	10.9 mg/g	89.9 ± 1.3 (n = 6)
	Mg	1.56 mg/g	76.9 ± 0.4 (n = 6)
	P	10.9 mg/g	73.3 ± 1.2 (n = 6)
	S	7.98 mg/g	80.0 ± 1.1 (n = 6)

c) Examples of results of the analysis of samples from the Environmental Specimen Bank

Sample	Element	Concentration
Pine shoots Dübener Heide 2006 (0210/0/0306/11200/0)	Ba	3.37 ± 0.03 µg/g (n = 6)
	Fe	58.6 ± 2.2 µg/g (n = 6)
	Mg	0.722 ± 0.039 mg/g (n = 6)
	Mn	405 ± 4 µg/g (n = 6)
	S	1.05 ± 0.03 mg/g (n = 6)
	Zn	45.7 ± 1.50 µg/g (n = 6)
Spruce shoots Berchtesgaden 2006 (0110/0/0506/01201/0)	Ba	9.81 ± 0.09 µg/g (n = 6)
	Fe	32.7 ± 1.2 µg/g (n = 5)
	Mg	1.24 ± 0.01 mg/g (n = 5)
	Mn	104 ± 2 µg/g (n = 6)
	S	0.738 ± 0.008 mg/g (n = 5)
	Zn	48.0 ± 0.89 (n = 5)
Beech leaves Solling 2005 (0410/0/0805/14102/0)	Ba	20.3 ± 0.1 µg/g (n = 5)
	Fe	88.3 ± 1.1 µg/g (n = 6)
	Mg	1.22 ± 0.01 mg/g (n = 6)
	Mn	1270 ± 3 µg/g (n = 6)
	S	1.31 ± 0.01 mg/g (n = 6)
	Zn	20.4 ± 0.4 µg/g (n = 5)
Bladder wrack Eckwarderhörne 2005 (4000/0/0005/07302/0)	Ba	27.7 ± 0.3 µg/g (n = 6)
	Fe	560 ± 5 µg/g (n = 5)
	Mg	9.20 ± 0.06 mg/g (n = 5)
	Mn	255 ± 2 µg/g (n = 5)
	S	26.9 ± 0.2 mg/g (n = 5)
	Zn	75.0 ± 0.5 µg/g (n = 6)
Earthworm Saartal 2005 (2211/0/1005/02100/0)	Ca	7.22 ± 0.27 mg/g (n = 6)
	K	11.1 ± 0.1 mg/g (n = 6)
	Mg	1.28 ± 0.01 mg/g (n = 6)
	P	9.06 ± 0.11 mg/g (n = 6)
	S	7.43 ± 0.08 mg/g (n = 6)