HEAVY METALS, NITROGEN AND POPs IN EUROPEAN MOSSES: 2015 SURVEY

MONITORING MANUAL

International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops

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Working Group on Effects
of the
Convention on Long-range Transboundary Air Pollution

UNITED NATIONS ECONOMIC COMMISSION FOR EUROPE CONVENTION ON LONG-RANGE TRANSBOUNDARY AIR POLLUTION

MONITORING OF ATMOSPHERIC DEPOSITION OF HEAVY METALS, NITROGEN AND POPs IN EUROPE USING BRYOPHYTES

MONITORING MANUAL

2015 SURVEY

ICP Vegetation

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In collaboration with the participants

1. INTRODUCTION

The UNECE ICP VEGETATION

In the late 1980's, the International Cooperative Programme on the effects of air pollution on natural vegetation and crops (ICP Vegetation, formally ICP Crops) was established to consider the underlying science for quantifying damage to plants by air pollutants. Scientists from more than 40 countries currently participate in the ICP Vegetation (Harmens et al., 2013b). The programme is led by the UK and coordinated by the Centre for Ecology and Hydrology (CEH) in Bangor. Coordination of the European moss survey is since 2014 led by the Joint Institute for Nuclear Research (JINR) in Dubna, Russian Federation.

The programme is part of the activities of the Working Group on Effects (WGE) under the Convention on Long-Range Transboundary Air Pollution (LRTAP), which covers the UNECE (United Nations Economic Commission for Europe) region of Europe and North America. The ICP Vegetation is one of several ICPs and Task Forces investigating effects of pollutants on waters, materials, forests, ecosystems, health, and mapping their effects in the ECE region. International cooperation to control pollution is strengthened by the LRTAP Convention. Its Protocols commit countries to reducing pollutant emissions by specific target years. Results from the ICPs are used in both the development of these Protocols and in monitoring their success in reducing the impacts of air pollutants on health and the environment. For further information on the LRTAP Convention, WGE, and other ICPs, please visit the web pages listed in Annex 1.

Monitoring long-term and large-scale changes in heavy-metal deposition

Increased and excessive accumulation of heavy metals in the soil, ground water and organisms can cause retarded growth of trees and crops and increased levels of heavy metals in the food chain leading to man.

It is apparent that some heavy metals emitted into the air from sources such as industries and power stations are mainly spread locally around the emission source. The affected area might have a diameter of 10-50 km, depending on wind patterns and height of stacks. Examples of this kind of distribution are chromium and nickel. Other metals are transported longer distances due to the formation of a gaseous phase during combustion, leading to very small and easily transported particles. This appears to be the case with arsenic, cadmium, lead, mercury and zinc. The LRTAP Convention has negotiated the Heavy Metal Protocol in 1998 in Aarhus (Denmark), committing parties to reducing emissions and consequent long-range transport of heavy metals: the Protocol on Heavy Metals was amended in 2012. Further information is needed on the concentrations of heavy metals in the environment, deposition rates and pathways, and effects on human health and the environment. Data from the 2015 moss survey will add to that of previous European surveys in 1990, 1995, 2000, 2005 and 2010 (Harmens et al., 2010, 2013c; Schröder et al., 2010b), and thus will provide further information on temporal and spatial trends of concentrations of heavy metals in mosses in Europe at a high spatial resolution.

Mosses as biomonitors of atmospheric deposition of heavy metals

Anyone who wants to measure the fallout of heavy metals from the atmosphere has access to an alternative that is both simple and inexpensive as compared with the rather arduous methods of analysing precipitation with respect to metal concentrations. The dense carpets that *Hylocomium splendens*, *Pleurozium schreberi* and other pleurocarpous mosses form on the ground have turn out to be very effective traps of metals in precipitation and airborne particles. This allowed for a dense biomonitoring network to be established across Europe since 1990.

One of the main benefits to be gained from studying heavy-metal fallout through moss analyses is that metals are accumulated by the moss, leading to much higher concentrations than in air, rain and snow. The problems of contamination during sampling and analysis are therefore relatively small, and sampling can be carried out using relatively simple methods.

Mosses as biomonitors of atmospheric deposition of nitrogen

In the 2005 European moss survey, the total nitrogen concentration in mosses was determined for the first time. The spatial distribution of nitrogen concentrations in mosses appears to mirror atmospheric nitrogen deposition across Europe to a high degree and is potentially a valuable tool for identifying areas at risk from high atmospheric nitrogen deposition at a high spatial resolution (Harmens et al., 2011, 2013c; Schröder et al., 2010a). Determining the total nitrogen concentration in mosses in the 2015 survey would also allow investigation of temporal trends across Europe.

Mosses as biomonitors of atmospheric deposition of persistent organic pollutants (POPs)

In the 2010 European moss survey, selected POPs were determined in mosses for the first time in a pilot study in selected countries (Foan et al., 2014; Harmens et al., 2013b). Mosses have been applied in the past as biomonitors of POPs, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in particular, at the local or national scale (Harmens et al., 2013a). For the 2015 survey we suggest to extend the pilot study conducted in 2010 to other countries. We suggest to focus on PAHs, PCBs, polybromodiphenylethers (PBDEs), dioxins, and perfluorooctane sulfonic acid and its salts (PFOS), but other POPs could also be included if there is a national interest.

2. AIMS AND OBJECTIVES

The aims of the 2015 survey are to:

- Characterise qualitatively (and quantitatively where possible) the regional atmospheric deposition of heavy metals, nitrogen and POPs in Europe.

- Indicate the location of important heavy metal, nitrogen and POPs emission sources and the extent of particularly polluted areas.

- Produce maps of the deposition patterns of heavy metals and nitrogen (and possibly for selected POPs) for Europe and analyse spatial trends.

- Provide field-based evidence of the extent of long-range trans-boundary pollution in Europe.

- Analyse temporal trends to establish the effectiveness of air pollution abatement policies within Europe.

- Where possible, determine the effect of canopy drip on the concentration of heavy metals and nitrogen in mosses by comparing moss samples from open fields and adjacent forest stands (Kluge et al., 2013; Skudnik et al., 2014).

3. SAMPLING PROGRAMME

Number of sampling sites

Similar to previous surveys each country should aim to collect at least 1.5 moss samples/1000 km^2. If this is not feasible, a sampling density of at least two moss sample sites per EMEP[1] grid (50 km x 50 km) is recommended. It is recommended to make an even and objective distribution of the samples whenever possible, and to have a more dense sampling regime in areas where steep gradients in the deposition of heavy metals can be foreseen. To aid the analysis of temporal trends in the concentration of heavy metals in mosses, it is recommended to collect samples from the same sites as in the previous surveys. Regarding the determination of POPs, a lower sampling density is anticipated, depending on national resources available.

To assess a statistically valid number of sampling sites for a given region, country or landscape, one can also make use of measurement data from previous surveys and the following formula:

$$\text{Minimum sample size} = (1.96 * \text{standard deviation} / \text{error tolerance} * \text{Mean value})^2$$

For the error tolerance you may insert values between 0 (no tolerance) and 1 (full tolerance). It is most common to use either 0.1 or 0.2 (Garten et al., 2007; Qiu et al., 2001).

Moss species

Only pleurocarpous mosses should be sampled. As in earlier investigations two pleurocarpous moss species are favoured: *Hylocomium splendens* and *Pleurozium schreberi*. However, in some countries it might be necessary to use other pleurocarpous species. In that case, the first choice would be *Hypnum cupressiforme*, followed by *Pseudoscleropodium purum* (Harmens et al., 2010; 2013c). Among other moss species, *Abietinella abietina* var. *abietina* (Hedw.) M. Fleisch was successfully tested in mountainous areas (Zechmeister, 1998; 2003). In dryer, arid regions others species might need to be sampled if none of the preferred species are present, for example

[1] Co-operative programme for monitoring and evaluation of the long-range transmission of air pollutants in Europe. http://www.emep.int/

Barbula indica (Nguyen Viet et al., 2010), *Camptothecium lutescens* and *Homalothecium sericeum* (Barandovski et al., 2008). The use of bryophytes other than *Hylocomium* or *Pleurozium* must be preceded by a comparison and calibration of their uptake of heavy metals relative to the main preferred species. For correct nomenclature of moss species we refer to Hill et al. (2006). Ignatov et al. (2006) and Stepanov (2003) provided a checklist of moss species for Eastern Europe and North Asia.

Field sampling

Sampling in the field should be done according to the following principles:

1. Each sampling point should be situated at least 3 m away from the nearest projected tree canopy: in gaps of forests (diameter >10 m) or plantations primarily (diameter >5 m), without pronounced influence from canopy drip from trees, preferably on the ground or on the surface of decaying stumps. In nitrogen polluted regions with high density of livestock the horizontal distances to tree crowns should be more than 7 m (Mohr, 2014).

2. In habitats such as open heathland, grassland or peatland, sampling below a canopy of shrubs or large-leafed herbs should be avoided, as well as areas with running water on slopes.

3. Coarse contamination of moss samples (litter, soil animals) should carefully removed. Moss cushions that are sandy and/or occupied by ants should be avoided.

4. The sampling points should be located at sites representative of non-urban areas of the respective countries. In remote areas the sampling points should be at least 300 m from main roads (highways), villages and industries and at least 100 m away from smaller roads and houses.

5. In order to enable comparison of the data from this survey with previous surveys, it is suggested to collect moss samples from the same (or nearby, i.e. no more than 2 km away but with the same biotope conditions) sampling points as used in the most recent moss surveys. In addition, sampling of mosses near (long-term) monitoring stations of atmospheric heavy metal, nitrogen or POPs deposition (e.g. national or EMEP deposition measurement stations) is recommended in order to directly compare their concentration in moss with the accumulated atmospheric deposition.

6. It is recommended to make one composite sample from each sampling point, consisting of five to ten (ten for POPs) subsamples, if possible, collected within an area of about 50 m x 50 m.

   Mosses: In the composite sample only one moss species should be represented. The sub-samples should be placed side by side or on top of each other in suitable containers. For metal and nitrogen analysis use large paper or plastic bags, tightly closed to prevent contamination during transportation. The amount of fresh moss needed for metal analysis is about one litre.

For POP analysis use pre-heated (450 ^{o}C, 6 hrs) glass jars. To protect the sampled moss from the rubber coatings inside the lid, cover the opening of the glass jar with a piece of aluminium foil before closing the lid. The amount of moss needed for POP analysis is about one litre. As some POPs are susceptible to volatilization and photochemical breakdown, samples for POPs analysis should be kept cool and in the dark at all times. Note: The latter is less important when analysing only for the seven PAHs recommended by the EU (see annex 4).

7. Smoking is forbidden during sampling and further handling of samples, and disposable plastic, non-talcum gloves should be used when picking up the moss for metal or nitrogen analysis. Do not use vinyl examination gloves if they are powdered with talcum as this will contaminate the samples. When sampling moss for analysis of POPs avoid using latex gloves as these can cause matrix problems and use nitrile gloves instead. Preferably use bare hands, but wash them thoroughly with copious amounts of *Sphagnum* (peat moss) before sampling moss for POPs.

8. Samples should preferably be collected during the period April − October. In arid regions of Europe it is advised to collect the samples during the wet season. Although the heavy metal concentrations in *Hylocomium splendens* and *Pleurozium schreberi* appear not to vary with season (Thöni et al., 1996, Berg and Steinnes, 1997), this might not be true for other moss species (e.g. Boquete et al., 2011; Couto et al., 2003; Zechmeister et al., 2003) and all climates in Europe. Therefore, it is suggested to sample the mosses in the shortest time window possible.

9. Each locality must be given co-ordinates, preferably longitude and latitude (Greenwich co-ordinates, 360º system), suitable for common data processing.

10. In order to determine the variability associated with the entire procedure (sampling + analysis), multiple moss samples (at least 3 samples per site) must be collected from at least two sites with different levels of overall contamination (one expected to have a high level of contamination and one expected to have a low level of contamination based on the results of the 2010 survey). These multiple moss samples must be collected, processed and analysed individually in order to characterise the variability of the data.

4. ANALYTICAL PROGRAMME

Utmost care should be taken in order to avoid contamination from smoke and laboratory tables. The material should therefore be handled on clean laboratory paper, glass shields, or clean polythene. Non-talcum, disposable plastic gloves should be worn and no metal tools should be used. For POP analysis, nitrile gloves are recommended, do not use latex gloves.

Cleaning and storing of moss samples

If the samples cannot be cleaned straight after sampling, they should be put into paper bags and dried and stored at room temperature (20-25 ^{o}C) until further treatment. Alternatively, samples can be deep-frozen.
For POPs analysis, samples should be stored at -20 ^{o}C and in the dark (see Field sampling – point 6). For PAH analysis it may not be necessary to freeze the samples.

However, it is desirable to use the same samples for determination of other POPs such as HCB, which are more volatile than the PAH.

The samples should be carefully cleaned from all dead material and attached litter, so that just the green and green-brown shoots from the last three years growth are included. Brown parts should not be included, even if the green parts only represent the last two to three years of growth.

Drying of moss samples before determination of heavy metals and nitrogen

The samples should be dried to constant weight at 40 ºC, which is used as a reference for the calculations. It is recommended to record the drying loss at 40 ºC (compared to room temperature) for future reference. The rest of the dried material not used in analyses should be stored in an environment specimen bank for future investigations.

For mercury, analysis should be conducted on fresh material or material dried at a lower temperature than 40 ºC and the determination of drying loss at 40 ºC on a separate aliquot is recommended.

Drying of moss samples before determination of POPs

Preparations of the moss samples for the determination of POPs will depend on the compounds analysed and the analytical technique applied in the laboratory. For example, drying of moss samples for the determination of PAHs might be best done by freeze-drying (lyophilisation). However, laboratories need to check for losses of POPs in the various steps leading up to the analysis. The feasibility of determining POPs concentrations in one lab for all samples collected across Europe should be investigated.

Determination of heavy metals

Digestion
Wet ashing of a homogeneous sub-sample is recommended for the decomposition of organic material. Dry ashing is not acceptable. The preferred method of decomposition is microwave digestion. Wet ashing, using nitric acid, has been used in most countries in the past and has proven to give reproducible results. If excess acid is evaporated, samples should not be allowed to become completely dry. **Note:** wet ashing should not be applied when Instrumental Neutron Activation Analysis (INAA) is used as analytical technique; a homogenous, dried sub-sample should be analysed without further pre-treatment.

Analytical technique
The metal determinations can be performed using various analytical techniques, however ICP-ES/MS (inductively coupled plasma emission/mass spectroscopy) and INAA are the preferred methods (Harmens et al., 2013c). It should be noted that INAA tends to give higher metal concentrations as it determines the total heavy metal concentration (Steinnes et al., 1993). Therefore, it is recommended to compare the results for INAA with other techniques such as ICP-ES/MS using the same moss samples and include standard moss reference material to further compare the performance of these techniques (see below).

An intercalibration of the analytical procedure took place in recent European moss surveys. For quality assurance purposes, participants must include again the moss standards M2 and M3 that were used in recent surveys (Steinnes et al., 1997; Harmens et al., 2010). The moss standards must be analysed at the same time as the collected moss samples. The moss standards will be supplied by Juha Piispanen (Juha.Piispanen@metla.fi), Finnish Forest Research Institute, Oulu Research Unit. The following certified reference material of organic contamination has to be included for POPs: IAEA-140/OC Fucus (35g) from Analab (seaweed material containing organochlorine compounds (pesticides and PCBs) and petroleum hydrocarbons (aliphatic hydrocarbons and PAHs)).

For quality assurance and cross-border calibration purposes, participants are encouraged to exchange ca. six to ten moss samples (clean and three years growth selected) from selected sites near the border of the country with neighbouring countries.

Current analytical techniques allow multi-element analysis; therefore participants are encouraged to report data on as many elements as possible. If this is not feasible, participants should at least report data for the elements that were included in previous European moss surveys, i.e. Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sb, V, and Zn. Including as many elements as possible will aid the identification of the sources of heavy metals by applying multivariate analysis. For example, Al and Sb were added to the list in 2005 in order to assess the possible soil particle contribution (Al) and as an indication of enrichment in the urban atmosphere due to the use in automobile brake lining (Sb).

Determination of nitrogen

The ICP Vegetation encourages participants also to determine the total nitrogen concentration in mosses and hopes to increase the spatial coverage of Europe in comparison to previous surveys (Harmens et al., 2011). To directly compare the nitrogen concentration in mosses with atmospheric nitrogen deposition, it is recommended to include sites near monitoring stations of atmospheric nitrogen deposition. Suggested methods for nitrogen analysis are Kjeldahl (wet digestion) and elemental analysis (Dumas method). For quality assurance purposes the nitrogen concentration in the moss standards M2 and M3 must be determined (in addition to any certified standards for nitrogen) along with the moss samples (see above). Recommended values for M2 and M3 for nitrogen were reported previously (Harmens et al., 2010).

Determination of POPs

The ICP Vegetation encourages participants also to determine the concentration of selected POPs. Annex 4 provides a list of POPs recommended to be included. No specific analytical techniques are recommended at this stage due to the diverse nature of POPs. However, participants are encouraged to investigate the possibility of analysing POPs in one certified laboratory to reduce variability due to laboratory differences. The Norwegian Institute for Air Research (NILU) is willing to analyse POPs in mosses sampled across Europe. For further details on costs etc. please contact Hilde Thelle Uggerud at htu@nilu.no

Optional: Determination of long-lived radionuclides

The Moss Survey Coordination Centre is also interested in receiving data on concentrations of long-lived radionuclides in mosses, for example Pb-210 and Cs-137. However, data for Cs-137 will have to be interpreted with care as monitoring of recently deposited Cs-137 might be confounded by Cs-137 deposited a long time ago (e.g. fallout due to the Chernobyl accident) as Cs-137 is a mobile radionuclide in ecosystems. Low background gamma-ray spectrometry can be provided by several interested laboratories in Russia, Kazakhstan and Serbia. One should bear in mind that the necessary amount of dry moss for measurement may be much larger than for simple elemental determination: typically 10-12 g of dry moss.

5. FURTHER SITE-SPECIFIC DATA

To determine which site-specific parameters affect the heavy metal, nitrogen and POPs concentration in mosses, participants are encouraged to provide further site-specific data via Web MossMet. This will allow detailed geostatistical analysis of factors influencing element concentrations in mosses (Schröder et al., 2010a,b). For further details, please contact Mr Winfried Schröder (wschroeder@iuw.uni-vechta.de).

6. DATA PROCESSING, REPORTING AND PUBLICATION

The Moss Survey Coordination Centre (Dubna, Russian Federation) will be responsible for common data processing, the construction of maps, and the final report. The ICP Vegetation Programme Coordination Centre will take on an advisory role and will coordinate communication with and dissemination of results to the Working Group on Effects of the LRTAP Convention. Detailed geostatistical analysis of data provided to MossMet will be conducted by Mr Winfried Schröder and colleagues (Germany) in close collaboration with the participants and the Moss Survey Coordination Centre.

All data should be sent to Ms Marina Frontasyeva, Moss Survey Coordination Centre (see front page for details). Please submit the data by e-mail as an Excel spreadsheet to mfrontasyeva@jinr.ru

THE SPREADSHEET SHOULD CONTAIN THE FOLLOWING INFORMATION (see Annex 2):

Country

Name, address, telephone no. and e-mail address for all participants

Analytical procedure used for each metal, nitrogen, POPs and radionuclides, including sample preparation, digestion method, and analytical technique.

Regarding important metadata, please provide at least the following data in rows, with one row for each site sampled. The column headings should read:

Site name

Coordinates (in degrees)

Date sampled

Altitude (m above sea level)

Land cover (according to CORINE classification label level 3; see Annex 3)

Topography (plain or slope)

Distance (m) to the nearest projection of the tree canopy. If moss was sampled under tree canopy, please fill in distance of 0 m. If the distance was more than 10 m, please fill in >10 m. Report accurately in m if distance between 0 and 10 m.

Any further details regarding the site or climate are optional

Moss species (see Hill et al., 2006)

In addition, please make use of the new web form with additional metadata. One may use a corresponding print for documentation in the field (Formular.htm), an explanation of all metadata is provided in Annex 5. For further information, please contact Winfried Schröder (winfried.schroeder@uni-vechta.de).

For each metal, nitrogen, each POP and each radionuclide the name and units of concentration must be listed. For each metal, nitrogen, each POP and radionuclide the quantification limit of the applied analytical technique must be provided.

Data must also include the individual values (metals, N, POPs, radionuclide) for each moss standard, such that the mean value and standard deviations per moss standard can be determined for each participating laboratory. In addition, data for cross-border calibration should be clearly labelled.

A report will be prepared in 2018 that will contain European maps of heavy metal, nitrogen and POPs concentrations in mosses and wherever possible, an indication of temporal trends.

7. TIME SCHEDULE

The main sampling period will be April to October 2015 (or 2016, depending on available funding). Data should be submitted to the Moss Survey Coordination Centre as soon as possible, but no later than 1 September 2016 (or 1 April 2017 if survey conducted in 2016). It is envisaged that preliminary maps will be produced by September 2017, and that a final report will be prepared by the summer of 2018.

8. FUNDING

Sampling and analyses must be paid for by each country separately. Coordination and collating data by the ICP Vegetation Moss Survey Coordination Centre will be funded by the JINR, Dubna, Russian Federation.

REFERENCES

Barandovski, L., Cekova, M., Frontasyeva, M.V., Pavlov, S.S., Stafilov, T., Steinnes, E., Urumov, V. (2008). Atmospheric deposition of trace element pollutants in Macedonia studied by the moss biomonitoring technique. Environmental Monitoring and Assessment 138: 107-118.

Berg, T., Steinnes, E. (1997) Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute values. Environmental Pollution 98: 61-71.

Boquete, M.T., Fernandez, J.A., Aboal, J.R., Carballeira, A. (2011). Analysis of temporal variability in the concentrations of some elements in the terrestrial moss *Pseudoscleropodium purum*. Environmental and Experimental Botany 72: 210–216.

Couto, J.A., Fernandez, J.A. , Aboal, J.R., Carballeira, A. (2003). Annual variability in heavy-metal bioconcentration in moss: sampling protocol optimization. Atmospheric Environment 37: 3517–3527.

Garten, C.T., Kang, S., Brice, D.J, Schadt, C.W., Zhou, J. (2007). Variability in soil properties at different spatial scales (1 m – 1 km) in a deciduous forest ecosystem. Soil Biology and Biochemistry 39: 2621-2627.

Foan, L., Leblond, S., Thöni, L., Raynaud, C., Santamaria, J.M, Sebilo, M., Simon, V. (2014). Spatial distribution of PAH concentrations and stable isotopic signatures ($\delta^{13}C$, $\delta^{15}N$) in mosses from three European areas - Characterization by multivariate analysis. Environmental Pollution 184: 113-122.

Harmens, H., Foan, L., Simon, V., Mills, G. (2013a). Terrestrial mosses as biomonitors of atmospheric POPs pollution: A review. Environmental Pollution 173: 245-254.

Harmens, H., Mills, G., Hayes, F., Norris, D. and the participants of the ICP Vegetation. (2013b). Air pollution and vegetation. ICP Vegetation annual report 2012/2013. ICP Vegetation Programme Coordination Centre, CEH Bangor, UK.

ISBN: 978-1-906698-43-0. http://icpvegetation.ceh.ac.uk

Harmens, H., Norris, D. A., Cooper, D.M., Mills, G., Steinnes E., Kubin, E., Thöni, L., Aboal, J.R., Alber, R., Carballeira, A., Coşkun, M., De Temmerman, L., Frolova, M., Gonzáles-Miqueo, L., Jeran, Z., Leblond S., Liiv, S., Maňkovská, B., Pesch, R., Poikolainen, J., Rühling, Å., Santamaria, J. M., Simonèiè, P., Schröder, W., Suchara, I., Yurukova, L., Zechmeister, H. G. (2011). Nitrogen concentrations in mosses indicate the spatial distribution of atmospheric nitrogen deposition in Europe. Environmental Pollution 159: 2852-2860.

Harmens, H., Norris, D., Mills, G., and the participants of the moss survey (2013c). Heavy metals and nitrogen in mosses: spatial patterns in 2010/2011 and long-term temporal trends in Europe. ICP Vegetation Programme Coordination Centre, CEH Bangor, UK. ISBN: 978-1-906698-38-6.
http://icpvegetation.ceh.ac.uk

Harmens, H., Norris, D.A., Steinnes, E., Kubin, E., Piispanen, J., Alber, R., Aleksiayenak, Y., Blum, O., Coşkun, M., Dam, M., De Temmerman, L., Fernández, J.A., Frolova, M., Frontasyeva, M., González-Miqueo, L., Grodzińska, K., Jeran, Z., Korzekwa, S., Krmar, M., Kvietkus, K., Leblond, S., Liiv, S., Magnússon, S.H., Maňkovská, B., Pesch, R., Rühling, Å., Santamaria, J.M., Schröder, W., Spiric, Z., Suchara, I., Thöni, L., Urumov, V., Yurukova, L., Zechmeister, H.G. (2010). Mosses as biomonitors of atmospheric heavy metal deposition: spatial and temporal trends in Europe. Environmental Pollution 158: 3144-3156.

Hill, M.O., Bell, N., Bruggeman-Nannenga, M.A., Brugués, M., Cano, M.J., Enroth, J., Flatberg, K.I., Frahm, J.-P., Gallego, M.T., Garilleti, R., Guerra, J., Hedenäs, L., Holyoak, D.T., Hyvönen, J., Ignatov, M.S., Lara, F., Mazimpaka, V., Muñoz, J., Söderström, L. (2006). An annotated checklist of the mosses of Europe and Macaronesia. Journal of Bryology 28: 198-267.

Ignatov, M.S., Afonina, O.M., Ignatova, E.A. (2006). Check-list of mosses of East Europe and North Asia. Arctoa 15: 1–130. http://arctoa.ru/ru/Archive-ru/15/Checklist.pdf (in Russian).

Kluge, M., Pesch, R., Schröder, W., Hoffmann, A. (2013). Accounting for canopy drip effects of spatiotemporal trends of concentrations of N in mosses, atmospheric N depositions and critical load exceedances: a case study from North-Western Germany. Environmental Sciences Europe 25: 26.

Mohr, K. (2014). Biomonitoring von Stickstoffdepositionen mit Moosen. (Biomonitoring of nitrogen deposition with mosses). Gefahrstoffe Reinhaltung der Luft 6/2014.

http://www.gefahrstoffe.de/gest/article.php?data[article_id]=79012

Nguyen Viet, H., Frontasyeva, M.V., Trinh Thi, T.M., Gilbert, D., Bernard, N. (2010). Atmospheric heavy metal deposition in Northern Vietnam: Hanoi and Thainguyen case study using the moss biomonitoring technique, INAA and AAS. Environmental Science and Pollution Research 17: 1045-1052.

Qiu, Y., Fu, B., Wang, J., Chen, L. (2001). Spatial variability of soil moisture content and its relation to environmental indices in a semi-arid gully catchment of the Loess Plateau, China. Journal of Arid Environments 49:723-750.

Schröder, W., Holy, M., Pesch, R., Harmens, H., Fagerli, H., Alber, R., Coşkun, M., De Temmerman, L., Frolova, M., González-Miqueo, L., Jeran, Z., Kubin, E., Leblond, S., Liiv, S., Maňkovská, B., Piispanen, J., Santamaría, J.M., Simonèiè, P., Suchara, I., Yurukova, L., Thöni. L., Zechmeister, H.G. (2010a). First Europe-wide correlation analysis identifying factors best explaining the total nitrogen concentration in mosses. Atmospheric Environment 44: 3485-3491.

Schröder, W., Holy, M., Pesch, R., Harmens, H., Ilyin, I., Steinnes, E., Alber, R., Aleksiayenak, Y., Blum, O., Coşkun, M., Dam, M., De Temmerman, L., Frolova, M., Frontasyeva, M., González-Miqueo, L., Grodzińska, K., Jeran, Z., Korzekwa, S., Krmar, M., Kubin, E., Kvietkus, K., Leblond, S., Liiv, S., Magnússon,S., Maňkovská, B., Piispanen, J., Rühling, Å., Santamaria, J., Spiric, Z., Suchara, I., Thöni, L., Urumov, V., Yurukova, L., Zechmeister, H.G. (2010b). Are cadmium, lead and mercury concentrations in mosses across

Europe primarily determined by atmospheric deposition of these metals? Journal of Soils and Sediments 10: 1572-1584.

Skudnik, M., Jeran, Z., Batic, F., Simončič, P., Lojen, S., Kastelec, D. (2014). Influence of canopy drip on the indicative N, S and $\delta^{15}N$ content in moss *Hypnum cupressiforme*. Environmental Pollution 190: 27-35.

Steinnes, E., Johansen, O., Røyset, O., Ødegård, M. (1993). Comparison of different multielement techniques for analysis of mosses used as biomonitors. Environmental Monitoring and Assessment 25: 87-97.

Steinnes, E., Rühling, Å., Lippo, H, Makinen, A. (1997) Reference materials for large-scale metal deposition surveys. Accreditation and Quality Assurance 2: 243-249.

Stepanov, N.V. Higher spore plants. (2003), Krasnoyarsk State University, Krasnoyarsk: 1-180 (in Russian). http://elib.sfu-kras.ru/bitstream/2311/1424/1/up_sporplants.pdf

Thöni, L., Schnyder, N., Krieg, F. (1996) Comparison of metal concentrations in three species of mosses and metal freights in bulk precipitation. Fresenius Journal of Analytical Chemistry 354: 703-708.

Zechmeister H.G. (1998). Annual growth of four pleurocarpous moss species and their applicability for biomonitoring heavy metals. Environmental Monitoring and Assessment 52: 441-451.

Zechmeister, H.G., Hohenwallner, D., Riss, A., Hanus-Illnar, A. (2003) Variations in heavy metal concentrations in the moss species *Abietinella abietina* (Hedw.) Fleisch according to sampling time, within site variability and increase in biomass. Science of the Total Environment 301: 55-65.

Annex 1

WWW LINKS

ICP Vegetation	http://icpvegetation.ceh.ac.uk
Moss Survey Coordination Centre	http://flnp.jinr.ru/naa
UNECE	http://www.unece.org
LRTAP Convention	http://www.unece.org/env/lrtap/welcome.html
Working Group on Effects	http://www.unece.org/env/lrtap/WorkingGroups/wge/welcome.html This web page contains links to the other ICPs and the Task Force on Health.
EMEP	http://www.emep.int With links to MSC-West and MSC-East.

Stockholm Convention on persistent organic pollutants (POPs) http://chm.pops.int

Annex 2

Template data sheet

Country																												
For all participants:																												
Name																												
Address																												
Tel.																												
e-mail																												
Full description of analytical procedure for each metal, N and POP, including sample storage and preparation, digestion method and analytical technique																												
							Distance to nearest																				**Each**	**Any**
Site name	**Longitude**	**Latitude**	**Sample date**	**Altitude (m)**	**Land cover**	**Topography**	**tree canopy projection**	**Further details**	**Moss species**	**Al**	**As**	**Cd**	**Cr**	**Cu**	**Fe**	**Hg**	**Ni**	**Pb**	**Sb**	**V**	**Zn**	**Other metals**	**N**	**POP**	**radionuclide**
							(m)			**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(ug/g)**	**(mg/g)**	**(unit)**	**(unit)**
	xx°xx'xx"	xx°xx'xx"	dd/mm/yr		See annex 3	Plain or slope	Fill in 0 m if sampled under tree canopy	Site or climate																		
	or in decimals	or in decimals					Report accurately in m if distance between 0 and 10 m																			
							Fill in >10 m if sampling distance is >10 m																			
								Quantification limit for each metal																		
Also include:																										
Values for all moss standard runs (M2 & M3)																										
Data to determine overall variability (see Monitoring Manual, field sampling bullet point 10)																										
Also include cross-border calibration data (if done any)																										

Annex 3

Corine Land Cover 2000 classes

Code Level 3	Label Level 1	Label Level 2	Label Level 3
111	Artificial surfaces	Urban fabric	Continuous urban fabric
112	Artificial surfaces	Urban fabric	Discontinuous urban fabric
121	Artificial surfaces	Industrial, commercial and transport units	Industrial or commercial units
122	Artificial surfaces	Industrial, commercial and transport units	Road and rail networks and associated land
123	Artificial surfaces	Industrial, commercial and transport units	Port areas
124	Artificial surfaces	Industrial, commercial and transport units	Airports
131	Artificial surfaces	Mine, dump and construction sites	Mineral extraction sites
132	Artificial surfaces	Mine, dump and construction sites	Dump sites
133	Artificial surfaces	Mine, dump and construction sites	Construction sites
141	Artificial surfaces	Artificial, non-agricultural vegetated areas	Green urban areas
142	Artificial surfaces	Artificial, non-agricultural vegetated areas	Sport and leisure facilities
211	Agricultural areas	Arable land	Non-irrigated arable land
212	Agricultural areas	Arable land	Permanently irrigated land
213	Agricultural areas	Arable land	Rice fields
221	Agricultural areas	Permanent crops	Vineyards
222	Agricultural areas	Permanent crops	Fruit trees and berry plantations
223	Agricultural areas	Permanent crops	Olive groves
231	Agricultural areas	Pastures	Pastures
241	Agricultural areas	Heterogeneous agricultural areas	Annual crops associated with permanent crops
242	Agricultural areas	Heterogeneous agricultural areas	Complex cultivation patterns
243	Agricultural areas	Heterogeneous agricultural areas	Land principally occupied by agriculture, with significant areas of natural vegetation
244	Agricultural areas	Heterogeneous agricultural areas	Agro-forestry areas
311	Forest and semi natural areas	Forests	Broad-leaved forest
312	Forest and semi natural areas	Forests	Coniferous forest
313	Forest and semi natural areas	Forests	Mixed forest
321	Forest and semi natural areas	Scrub and/or herbaceous vegetation associations	Natural grasslands
322	Forest and semi natural areas	Scrub and/or herbaceous vegetation associations	Moors and heathland
323	Forest and semi natural areas	Scrub and/or herbaceous vegetation associations	Sclerophyllous vegetation
324	Forest and semi natural areas	Scrub and/or herbaceous vegetation associations	Transitional woodland-shrub
331	Forest and semi natural areas	Open spaces with little or no vegetation	Beaches, dunes, sands
332	Forest and semi natural areas	Open spaces with little or no vegetation	Bare rocks
333	Forest and semi natural areas	Open spaces with little or no vegetation	Sparsely vegetated areas
334	Forest and semi natural areas	Open spaces with little or no vegetation	Burnt areas
335	Forest and semi natural areas	Open spaces with little or no vegetation	Glaciers and perpetual snow
411	Wetlands	Inland wetlands	Inland marshes
412	Wetlands	Inland wetlands	Peat bogs
421	Wetlands	Maritime wetlands	Salt marshes
422	Wetlands	Maritime wetlands	Salines
423	Wetlands	Maritime wetlands	Intertidal flats
511	Water bodies	Inland waters	Water courses
512	Water bodies	Inland waters	Water bodies
521	Water bodies	Marine waters	Coastal lagoons
522	Water bodies	Marine waters	Estuaries
523	Water bodies	Marine waters	Sea and ocean

Annex 4

Recommended list of persistent organic pollutants (POPs)

			POPs	Stockholm			
Name/Synonym	**Group**	**EMEP modelled**	**Protocol**	**Convention**	**Notes**		
PCB	Polychlorinated biphenyls		x	x	Dielectric fluids in transformers, capacitors, coolants		
BDE-x	Polybromodiphenylether	BDE-28, 47, 99, 153		2009	Flame retardants		
HBB	Polybrominated biphenyls			2009	Flame retardants, see polybromodiphenylether		
HxCDD	Polychlorinated dibenzo-p-dioxins (PCDD) (Dioxins)	x	x	x	PVC production, industrial bleaching, incineration		
PFOS	Perfluorooctane sulfonic acid and its salts			2009	(Fluoro)Surfactant		
	PAHs						
Benzo(a)anthracene	EU, US EPA				Seven EU PAHs are non-volatile and the most toxic		
Benzo(j)fluoranthene	EU						
Benzo(b)fluoranthene	EU, POPs Protocol indicator, US EPA						
Benzo(k)fluoranthene	EU, POPs Protocol indicator, US EPA						
Benzo(a)pyrene	EU, POPs Protocol indicator, US EPA	x	x				
Dibenzo(a,h)anthracene	EU						
Indeno(1,2,3-cd)pyrene	EU, POPs Protocol indicator, US EPA						
Naphthalene	US EPA						
Acenaphthylene	US EPA						
Acenaphthene	US EPA						
Fluorene	US EPA						
Phenanthrene	US EPA						
Anthracene	US EPA						
Fluoranthene	US EPA						
Pyrene	US EPA						
Chrysene	US EPA						
Dibenzo(a,h,)anthracen	US EPA						
Benzo(g,h,i)perylen	US EPA						

Annex 5

Additional Information on the metadata variables of the MossMet questionnaire

!Please note that the metadata fields marked in bold are mandatory!
!In the metadata form these fields are marked with *!

For queries, please contact Winfried Schröder (wschroeder@iuw.uni-vechta.de)

- **Date**:
  Please provide the date of sampling as day/month/year.

- Time:
  Please provide the time of sampling as hh:mm.

- Name:
  Please note your name. When inserting the metadata form into the MossMet-application you will automatically be provided with a username that will then be documented here instead.

- **Country**:
  Please name your country. When inserting the metadata form into the MossMet application a list of countries will be provided from a dropdown list.

- Admin. district:
  This field may be handled as needed. You may insert information on any type of district like e.g. forest administrative units or regions of any type.

- **Site name:**
  Please provide a site name. We would strongly encourage you to combine the official abbreviation of your country or administrative subunit with unique numerical values. In Germany we combine an abbreviation of our federal states with a number (as in BB1 for Brandenburg Site #1).

- Weather:
  Please insert the weather during sampling, whether rainy, cloudy, sunny or nebulous. Provided you prefer another weather type you should choose ‘others’ and type in this weather type as free text.

- **Latitude and longitude:**
  Here, insert the geographical coordinates of the sampling area in decimal degrees. When having a GPS available this can be provided automatically. Otherwise you would have to depend on topographical maps.
  Please note that decimal degrees are needed and not degrees in degree/minutes/ seconds. Example: If have a latitude coordinate of 54°, 30 min this would correspond to 54.5 in decimal degrees. If you have any problems transferring your coordinates please contact us.

- **Elevation [m]:**
  Here, please insert the elevation in m above sea level. When having a GPS available this can be provided automatically. Otherwise you would have to depend on topographical maps or use digital elevation models.

- **Topography:**
  Please describe the topography of the sampling area in terms of the options *plain*, *slope* or *ridge*. When inserting the metadata information into the digital questionnaire you will only have these three opportunities to choose from.

- Slope gradient [°]:
  If the sampling site is located on a slope please try to estimate the slope gradient in degrees.

- Direction:
  If the sampling site is located on a slope please provide information on the respective direction in terms of N, NNE, NE, ENE, E, ESE, SE, SSE, S, SSW, SW, WSW, W, WNW, NW, NNW, N. When inserting the metadata information into the digital questionnaire you will only have these options available.

- **Number of subsamples:**
  Please give the number of subsamples you took from the (50 * 50 m² sized) sampling area in order to get one mixed moss sample for the site.

- **Sampled moss species**:
  Name the moss species you sampled. If the moss species is not included in the list please enter the name of the moss species under 'others'.

- **Sampling from**:
  Please specify whether you took the moss from the ground or from dead wood / tree stump.

- Sampling volume [l]:
  Please specify how much moss material was collected in litres.

- Shoot length [cm]:
  If possible please specify the shoot length of the sampled moss material.

- **Growth type:**
  Please specify the growth type of the moss as either *sparse*, *single cushions* or *mats*.

- **Frequency:**
  Please specify the frequency of occurrence of the sampled moss in the sampling area as either *rare* or *frequent*.

- **Visible dust particles:**
  If you detected any visible dust (e.g. chalk particles) on the moss please provide corresponding information in terms of either *none*, *rare* or *numerous*.

- **Distance to tree crown projection:**
  Please specify the distance of the sampling area to the outer projection of tree crowns (and not tree trunks). Since according to the guideline more than one subsample is to be collected, please specify the minimum and maximum distances of all subsamples to the respective nearest tree crown projection. Furthermore, try to estimate the average distance of all subsamples to the nearest tree crown projection.
  If there are no trees in the nearer surrounding of the sampling area please leave this field blank. Accordingly

If you will not provide any information on the distance to tree crowns we will automatically assume that no trees are in the nearer surrounding of the sampling area. If you therefore leave this field blank a certain threshold distance value will then be generated automatically in the database. We will then handle the respective site as 'trees lying more than this distance away from the sampling area'. As a threshold distance we chose 200 m[1].

- **Distance to shrub**:
  Please specify the distance of the sampling area to shrubs. Proceed as you did in case of the trees.
  If you will not provide any information on the distance to shrubs we will automatically assume that no shrubs are in the nearer surrounding of the sampling area. If you therefore leave this field blank a certain threshold distance value will then be generated automatically in the database. We will then handle the respective site as 'shrubs lying more than this distance away from the sampling area'. As a threshold distance we here chose 20 m.

- **Tree / shrub layer height:**
  Please give an estimate on the shrub and tree layer height. Like above, try to specify the minimum, maximum and average height.

- **Coverage of trees around the site [%]:**
  Try to estimate the coverage of trees in the surroundings of the sampling area.

- **Coverage of shrubs around the site [%]:**
  Try to estimate the coverage of shrubs in the surroundings of the sampling area.

- Humus layer [cm]:
  Please specify the thickness of the humus layer in cm.

- Humus species:
  Please specify the type of humus species in terms of *duff, mildew, duff/mull* or *not specified.*

- Bedrock type:
  Please give information on the bedrock type and type in the scientific name as plain text. Examples for typical bedrock types would be *crystalline, crystalline igneous rock, crystalline metamorphic rock, limestone, sandstone, clay stone, loose sediment*.

- Soil texture:
  Please give information on the soil texture type in terms of *clay, silt, sand, loam, silty clay, sandy clay, clay loam, silt loam, sandy loam, loamy sand, silty clay loam, sandy clay loam*. You will only have the opportunity to insert information according to these possibilities.

[1] We assume that if the distance of the trees is greater than the threshold distance that either no further influence can longer be claimed or that the respective effect is overlaid by regional deposition patterns. According to recommendations of the World Metereological Organization (WMO) the distance from a precipitation measurement to any object should be more than twice its height. A 200 m threshold distance to trees should therefore be more than adequate.

- Soil type:
  If possible, please give information on the soil type. Due to the different existing classification schemes this field will be handled as a free text option.

- Main wind direction (due to epiphytic growth):
  If possible, please provide information on the main wind direction due to epiphytic growth in terms of N, NE, E, SE, S, SW, W, NW, N. When inserting the metadata information into the digital questionnaire you will only have these options available.

- **Surrounding land use:**
  Please choose one of the listed land use types that are characteristic for the sampling site and its surroundings. If none of these types fit choose 'others' and type in the respective land use category.

- **Surrounding vegetation:**
  Please choose one of the listed vegetation types that are characteristic for the sampling site and its surroundings. If none of these types fit choose 'others' and type in the corresponding vegetation category.

- **Distance of the sampling site to ....:**
  If any of the listed 18 emission sources are located near the sampling area please specify the corresponding distance.
  If you will not provide any information on these distances we will automatically assume that no such emission sources are in the nearer surrounding of the sampling area. If you therefore leave this field blank a certain threshold distance value will then be generated automatically in the database. We will then handle the respective site as 'the emission source lying more than this distance away from the sampling area'[2]. This aspect is very important since it will have an effect on the output of the statistical analysis / evaluation. The threshold distances we chose have not been evaluated empirically but mostly rely on specifications provided by the European and German empirical sampling guidelines.We mostly chose these threshold distances as double the critical distances put down in these protocols.
  Depending on the emission source we defined the threshold distances as follows:
  none vegetated areas: 50 m
  agricultural areas: 300 m
  ploughed agricultural fields: 300 m
  animal farming units: 100 m
  single houses and villages: 300 m
  towns and cities: 600 m
  unsealed roads: 50 m
  small paved country roads: 100 m
  federal roads: 300 m
  railroad tracks: 300 m
  motorways: 600 m
  industries w. high chimneys: 10000 m
  small industries: 2000 m
  waste incineration facilities: 10000 m

[2] We assume that if the distance of the emission source is greater than the threshold distance that either no further influence can longer be claimed or that the respective effect is overlaid by regional deposition patterns.

dumping grounds: 2000 m
combustion energy plants: 10000 m
construction sites: 2000 m
gravel pits: 2000 m

- Type of industry:
  If any industries are near the sampling area please provide information on the type of industry.

- Distance of the sampling site to environmental monitoring station:
  If any other environmental monitoring stations are located near the sampling area please specify the corresponding distance.

- Type of environmental monitoring station:
  If any other environmental monitoring stations are near the sampling area please provide information on the corresponding type.

- Comments:
  If you were not able to document all that you think is necessary for the description of the site please add any additional comments here.

- Additional material:
  If you have additional digital material available that may be of help for the description of the site please upload this information here. Examples could be digital photos of the sampling area or available digital maps, e.g. in terms of screenshots.

Date*: time:

Name:

Country*:

Administ. district:

Site name*:

Weather:

- rainy
- sunny
- cloudy
- nebulous
- others (please specifiy)

Latitude [dez]*:

Longitude [dez]*:

Elevation [m]*:

Topography*:

Slope gradient [°]:

Direction:

Number of subsamples*:

Sampled moss species*:

- Pleurozium schreberi
- Hylocomium splendens
- Hypnum cupressiforme
- Pseudoscleropodium purum
- Thuidium abietinum
- others (please specifiy)

Sampling from*:

- ground
- tree stump

Sample volume [l]:

- < 1
- 1 - 2
- > 2

Shoot length [cm]:

- 5 < 10
- 10 - 15
- > 15

Growth type*:

- sparse
- single cushions
- mats

Frequency*:

- rare
- frequent

Visible dust particles*:

- none
- rare
- numerous

Distance to tree crowns*	Average		m	Min		m	Max		m
Distance to shrubs*	Average		m	Min		m	Max		m
Tree / shrub layer height*	Average		m	Min		m	Max		m

Coverage of trees [%]*	0	0 -25	25-50	50-75	75-100
Coverage of shrubs [%]*	0	0 -25	25-50	50-75	75-100

Humus layer [cm]:

Humus species:

- duff
- mildew
- duff/ mull
- not specified

Bedrock type:

Soil texture:

Soil type:

Main wind direction
(due to epiphytic growth)

Surrounding Land use*:

- Urbanised area
- Industrialised area
- Forests - mixed
- Forests - coniferous
- Forests - broad leaved
- Natural grasslands
- Meadows and pastures
- Agriculture (crops, vineyard, ...)
- Moors and heathland
- Sparsely vegetated area
- Inland marshes / salt marshes
- others (please specifiy)

Surrounding vegetation*:

- Clearing within coniferous forest
- Clearing within broad leaved forest
- Clearing within mixed forest
- Clearing within young forest plantation
- Heathland
- Grasslands
- others (please specifiy)

Distance of the sampling site to ....*

none vegetation areas	m	agricultural areas	m
animal farming units	m	ploughed agricultural fields	m
single houses	m	village	m
town	m	unsealed roads	m
small paved country roads	m	federal roads	m
motorways	m	railroad tracks	m
industries with high chimneys	m	small industries	m
waste incineration faculties	m	dumping grounds	m
combustion energy plants	m	construction sites	m
gravel pit	m		

Type of industry:

Distance of the sampling site to environmental monitoring station: m

Type of environmental monitoring station:

Comments:

Additional material: