HEAVY METALS, NITROGEN AND POPs IN EUROPEAN MOSSES: 2020 SURVEY

MONITORING MANUAL

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

http://flnp.jinr.ru/naa
http://icpvegetation.ceb.ac.uk
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

2020 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 approximately 50 countries currently participate in the ICP Vegetation. 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 air pollutants on waters, materials, forests, ecosystems, health, and mapping their effects in the ECE region. International cooperation to control air pollution is strengthened by the LRTAP Convention. Its Protocols commit countries to reducing air 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, geomorphology of the landscape, type of emission source and height of stacks. Examples of this kind of distribution are chromium and nickel (Steinnes and Frontasyeva, 1995; Steinnes et al., 2004). 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 2020 moss survey will add to that of previous European surveys in 1990, 1995, 2000, 2005, 2010, 2015 (Harmens et al., 2010, 2013b, 2015; Schröder et al., 2010b), and will provide further information on temporal and spatial trends of concentrations of heavy metals in mosses in Europe at a high spatial resolution. Data will contribute to assessment of the sufficiency and effectiveness of air pollution abatement policies in Europe.
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 compared with rather arduous methods of analysing precipitation with respect to metal concentrations. The dense carpets that *Hylocomium splendens*, *Pleurozium schreberi*, *Hypnum cupressiforme* and other pleurocarpous mosses form on the ground have turned 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. Sampling can be carried out using relatively simple methods avoiding contamination during sampling and analysis.

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, 2013b, 2014; Schröder et al., 2010a). Determining the total nitrogen concentration in mosses in the 2020 survey would also allow investigation of temporal trends across Europe since 2005.

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). 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 2020 survey, we suggest to extend the pilot studies conducted in 2010 and 2015 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.

Mosses as biomonitors of atmospheric deposition of microplastics

In the 2015 moss survey, Ireland conducted a pilot study using moss as biomonitors to estimate the atmospheric deposition of microplastics, microfibers in particular. Results of the study were presented at the 32nd ICP Vegetation Task Force meeting in Romania and at the meeting, other countries expressed an interest to conduct a similar pilot study in 2020.
2. **AIMS AND OBJECTIVES**

The aims of the 2020 survey are to:

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

- 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 transboundary pollution in Europe.

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

3. **SAMPLING PROGRAMME**

**Number of sampling sites**

Similar to previous surveys each country should aim to collect at least 1.5 moss samples/1000 km². If this is not feasible, a sampling density of at least two moss sample sites per ‘old’ 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 (Schröder et al., 2016):

\[
MSS = \left(1.96 \times \frac{\text{Stdev}}{\text{tol} \times \text{Mean}}\right)^2,
\]

- **MSS** = Minimum sample size
- **Stdev** = Standard deviation of measured element concentration in mosses
- **1.96** = Z-value, indicating significance level of 0.05
- **tol** = Error tolerance
- **Mean** = Mean value of measured element concentration in mosses

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).

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\(^1\) Co-operative programme for monitoring and evaluation of the long-range transmission of air pollutants in Europe. [http://www.emep.int/](http://www.emep.int/)
The above mentioned formula should be used for normally distributed data. For log-normal data, a modified formula should be applied (Schröder et al., 2016), and in case of not (log-)normal distributed data a Monte Carlo Simulation-based approach is recommended (Wosniok et al., 2019a). A software tool for applying this approach was published by Wosniok et al. (2019b).

**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 will 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; 2013b). Among other moss species, *Abietinella abietina* var. *abietina* (Hedw.) M. Fleisch was successfully tested in mountainous areas (Zechmeister, 1998; 2003). In dryer, arid regions sampling of other species might be needed if none of the preferred species are present, for example *Barbula indica* (Nguyen Viet et al., 2010), *Camptothecium lutescens*, *Homalothecium lutescens* and *Homalothecium sericeum* (Barandovski et al., 2008). 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. In habitats with trees, each sampling point should be located at least two times the height from the nearest tree if possible. In gaps of forests (diameter >10 m) or plantations (diameter > 5 m), without pronounced influence from canopy drip from trees, sampling points should be situated at least 3 m away from the nearest projected tree canopy, 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 be 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 (including industrial farming) and at least 100 m away from smaller roads (including unpaved 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 1
km away with comparable topographic 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 ten or more 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 °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).

For details on microplastics sampling, see Annex 5.

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.

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 and preferably at the end of the season. Sampling should involve no more than a small number of trained specialists and in large countries sampling might need to be split over two consecutive years.

9. Each locality must be given co-ordinates, preferably longitude and latitude (Greenwich co-ordinates, 360° 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 previous 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. For microplastics, further processing and analysis should be conducted in a clean air laboratory or fume hood as indoor air contains more micoplastics than outdoor air (see Annex 5).

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 ºC) until further treatment. Alternatively, samples can be deep-frozen. For POPs analysis, samples should be stored at –18 ºC 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 shoots from the last two-three years’ growth are included. Brown parts should not be included, even if the green parts only represent the last two years of growth.

Drying of moss samples before determination of heavy metals and nitrogen

The samples for analysis 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.

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., 2013b). 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 inter-calibration 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; 2015). 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 should be included for POPs: IAEA-459, which is available from the International Atomic Energy Agency (IAEA).

For quality assurance and cross-border calibration purposes, participants are encouraged to exchange ca. six to ten moss samples (clean and two - 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; 2015). 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 concentrations 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 mosses sampled across Europe for POPs. For further details on costs etc. please contact Hilde Thelle Uggerud at htu@nilu.no.

**Optional: Determination of microplastics**

Microplastics, which are plastic particles smaller than 5 mm, have gained increasing attention during the last decade owing to their ubiquity in natural environments. These waste plastics are either manufactured to be microscopic in size or come from plastic bottles, bags, clothing, etc., that have broken down and fragmented through UV radiation, physical abrasion and biodegradation. One of the most common types of microplastics are microfibres, which come from textiles, nets, fishing line and the fragmentation of larger plastic materials. Recently, several studies have focused on the atmospheric transport and deposition of microfibres (Dris et al., 2016; Cai et al., 2017; Allen et al., 2019; Stanton et al., 2019). The ICP Vegetation encourages participants to conduct a pilot study on using mosses as potential biomonitors of microplastics; their determination requires additional field, laboratory and quality control procedures, which are described in detail in Annex 5.

**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 (Fukushima accident in 2011) might be confounded by Cs-137 deposited a long time ago (e.g. fallout due to the Chernobyl accident in 1986) as Cs-137 is a mobile radionuclide in ecosystems. Low background gamma-ray spectrometry can be provided by several interested laboratories in the Russian Federation, 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 (Steinnes, 2016).
OPTIONAL: 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 (winfried.schroeder@uni-vechta.de).

DATA COLLECTION, PROCESSING AND REPORTING

The Moss Survey Coordination Centre (Dubna, Russian Federation) will be responsible for data collection, processing, the construction of maps, and the final report.

During moss sampling, we recommend that the Moss ICP Vegetation app for android phones (available from Google Play) is used to enter sampling site information automatically to the Data Management System (DMS). It automatically sets longitude and latitude of the sampling site, checks that the spelling of moss species is correct and allows attachment of photos of the moss samples and sampling sites. The app is integrated with the DMS (http://moss.jinr.ru) and all the information about sampling sites will be imported directly into the DMS.

Quality assured data on element and POP concentrations in mosses should be submitted (after chemical analyses of the samples) to the Data Management System (DMS) (Ososkov et al., 2016) of the UNECE ICP Vegetation. To gain access to the system, please, first register using the registration form on the website under the ‘login’ tab (http://moss.jinr.ru). The user manual is accessible after authorisation under the ‘help’ button. You can get an example of the correct file structures by clicking the relevant ‘Export’ button for sampling site data, heavy metals and nitrogen (‘Export datasets’), for POPs (‘Export POPs’) and moss reference material for interlaboratory comparison (‘Export Intercomparison’).

Participants will be able to upload three types of data files (see DMS for templates):
- Mandatory sampling site data;
- MossMet data (see Annex 2);
- Results of the chemical analyses.

In case of problems, please contact Dr. Marina Frontasyeva (marina@nf.jinr.ru), or Dr. Alexander Uzhinskiy (auzhinskiy@jinr.ru) at the Moss Survey Coordination Centre.

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.

The following data should be included in the DMS:

Country
Name, address, telephone no. and e-mail address for all participants
Analytical procedure used for each metal, nitrogen, POPs, microplastics 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 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 2. For further information, please contact Winfried Schröder (winfried.schroeder@uni-vechta.de).

For each metal, nitrogen, POP, microplastic and 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 2023 that will contain European maps of heavy metal, nitrogen and POPs concentrations in mosses and wherever possible, an indication of temporal trends.

5. **TIME SCHEDULE**

The main sampling period will be April to October 2020 (or 2021, depending on available funding and staff resources). Data should be submitted to the Moss Survey Coordination Centre as soon as possible, but no later than 1 September 2021 (or 1 April 2022 if survey conducted in 2021). It is envisaged that preliminary maps will be produced by December 2022, and that a final report will be prepared by the summer of 2023.
6. 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


Annex 1

Web 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

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 to be uploaded to DMS. The template data sheet can be downloaded from http://moss.jinr.ru

Comments template sheet:

- **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.

- **Country:**
  Please name your country.

- **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 (see https://www.worldatlas.com/aatlas/ctycodes.htm) or administrative subunit with unique numerical values.

- **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 liters.

- **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 m1.

- **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.
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 Meteorological 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.

- **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.

- **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 corresponding 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

---

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 Meteorological 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.
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 m². 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
crane 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.

Please note that the metadata fields marked in bold are mandatory!
In the Data sheet form these fields are marked with *!
For queries, please contact Winfried Schröder (wschroeder@iuw.uni-vechta.de)
### Annex 3

#### Corine Land Cover 2000 classes

<table>
<thead>
<tr>
<th>Code Level 3</th>
<th>Label Level 1</th>
<th>Label Level 2</th>
<th>Label Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>111</td>
<td>Artificial surfaces</td>
<td>Urban fabric</td>
<td>Continuous urban fabric</td>
</tr>
<tr>
<td>112</td>
<td>Artificial surfaces</td>
<td>Urban fabric</td>
<td>Discontinuous urban fabric</td>
</tr>
<tr>
<td>121</td>
<td>Artificial surfaces</td>
<td>Industrial, commercial and transport units</td>
<td>Industrial or commercial units</td>
</tr>
<tr>
<td>122</td>
<td>Artificial surfaces</td>
<td>Industrial, commercial and transport units</td>
<td>Road and rail networks and associated land</td>
</tr>
<tr>
<td>123</td>
<td>Artificial surfaces</td>
<td>Industrial, commercial and transport units</td>
<td>Port areas</td>
</tr>
<tr>
<td>124</td>
<td>Artificial surfaces</td>
<td>Industrial, commercial and transport units</td>
<td>Airports</td>
</tr>
<tr>
<td>131</td>
<td>Artificial surfaces</td>
<td>Mine, dump and construction sites</td>
<td>Mineral extraction sites</td>
</tr>
<tr>
<td>132</td>
<td>Artificial surfaces</td>
<td>Mine, dump and construction sites</td>
<td>Dump sites</td>
</tr>
<tr>
<td>133</td>
<td>Artificial surfaces</td>
<td>Mine, dump and construction sites</td>
<td>Construction sites</td>
</tr>
<tr>
<td>141</td>
<td>Artificial surfaces</td>
<td>Artificial, non-agricultural vegetated areas</td>
<td>Green urban areas</td>
</tr>
<tr>
<td>142</td>
<td>Artificial surfaces</td>
<td>Artificial, non-agricultural vegetated areas</td>
<td>Sport and leisure facilities</td>
</tr>
<tr>
<td>211</td>
<td>Agricultural areas</td>
<td>Arable land</td>
<td>Non-irrigated arable land</td>
</tr>
<tr>
<td>212</td>
<td>Agricultural areas</td>
<td>Arable land</td>
<td>Permanently irrigated land</td>
</tr>
<tr>
<td>213</td>
<td>Agricultural areas</td>
<td>Arable land</td>
<td>Rice fields</td>
</tr>
<tr>
<td>221</td>
<td>Agricultural areas</td>
<td>Permanent crops</td>
<td>Vineyards</td>
</tr>
<tr>
<td>222</td>
<td>Agricultural areas</td>
<td>Permanent crops</td>
<td>Fruit trees and berry plantations</td>
</tr>
<tr>
<td>223</td>
<td>Agricultural areas</td>
<td>Permanent crops</td>
<td>Olive groves</td>
</tr>
<tr>
<td>231</td>
<td>Agricultural areas</td>
<td>Pastures</td>
<td>Pastures</td>
</tr>
<tr>
<td>241</td>
<td>Agricultural areas</td>
<td>Heterogeneous agricultural areas</td>
<td>Annual crops associated with permanent crops</td>
</tr>
<tr>
<td>242</td>
<td>Agricultural areas</td>
<td>Heterogeneous agricultural areas</td>
<td>Complex cultivation patterns</td>
</tr>
<tr>
<td>243</td>
<td>Agricultural areas</td>
<td>Heterogeneous agricultural areas</td>
<td>Land principally occupied by agriculture, with significant areas of natural vegetation</td>
</tr>
<tr>
<td>244</td>
<td>Agricultural areas</td>
<td>Heterogeneous agricultural areas</td>
<td>Agro-forestry areas</td>
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<td>311</td>
<td>Forest and semi natural areas</td>
<td>Forests</td>
<td>Broad-leaved forest</td>
</tr>
<tr>
<td>312</td>
<td>Forest and semi natural areas</td>
<td>Forests</td>
<td>Coniferous forest</td>
</tr>
<tr>
<td>313</td>
<td>Forest and semi natural areas</td>
<td>Forests</td>
<td>Mixed forest</td>
</tr>
<tr>
<td>321</td>
<td>Forest and semi natural areas</td>
<td>Scrub and/or herbaceous vegetation associations</td>
<td>Natural grasslands</td>
</tr>
<tr>
<td>322</td>
<td>Forest and semi natural areas</td>
<td>Scrub and/or herbaceous vegetation associations</td>
<td>Moors and heathland</td>
</tr>
<tr>
<td>323</td>
<td>Forest and semi natural areas</td>
<td>Scrub and/or herbaceous vegetation associations</td>
<td>Sclerophyllous vegetation</td>
</tr>
<tr>
<td>324</td>
<td>Forest and semi natural areas</td>
<td>Scrub and/or herbaceous vegetation associations</td>
<td>Transitional woodland-shrub</td>
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<td>Forest and semi natural areas</td>
<td>Open spaces with little or no vegetation</td>
<td>Beaches, dunes, sands</td>
</tr>
<tr>
<td>332</td>
<td>Forest and semi natural areas</td>
<td>Open spaces with little or no vegetation</td>
<td>Bare rocks</td>
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<td>333</td>
<td>Forest and semi natural areas</td>
<td>Open spaces with little or no vegetation</td>
<td>Sparsely vegetated areas</td>
</tr>
<tr>
<td>334</td>
<td>Forest and semi natural areas</td>
<td>Open spaces with little or no vegetation</td>
<td>Burnt areas</td>
</tr>
<tr>
<td>335</td>
<td>Forest and semi natural areas</td>
<td>Open spaces with little or no vegetation</td>
<td>Glaciers and perpetual snow</td>
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<td>411</td>
<td>Wetlands</td>
<td>Inland wetlands</td>
<td>Inland marshes</td>
</tr>
<tr>
<td>412</td>
<td>Wetlands</td>
<td>Inland wetlands</td>
<td>Peat bogs</td>
</tr>
<tr>
<td>421</td>
<td>Wetlands</td>
<td>Maritime wetlands</td>
<td>Salt marshes</td>
</tr>
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<td>422</td>
<td>Wetlands</td>
<td>Maritime wetlands</td>
<td>Salines</td>
</tr>
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<td>Wetlands</td>
<td>Maritime wetlands</td>
<td>Intertidal flats</td>
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<td>Water bodies</td>
<td>Inland waters</td>
<td>Water courses</td>
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<td>Water bodies</td>
<td>Inland waters</td>
<td>Water bodies</td>
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<tr>
<td>521</td>
<td>Water bodies</td>
<td>Marine waters</td>
<td>Coastal lagoons</td>
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<td>Water bodies</td>
<td>Marine waters</td>
<td>Estuaries</td>
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<td>523</td>
<td>Water bodies</td>
<td>Marine waters</td>
<td>Sea and ocean</td>
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### Annex 4

**Recommended list of persistent organic pollutants (POPs)**

<table>
<thead>
<tr>
<th>Name/Synonym</th>
<th>Group</th>
<th>EMEP modelled</th>
<th>POPs</th>
<th>Stockholm</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB</td>
<td>Polychlorinated biphenyls</td>
<td>x</td>
<td>x</td>
<td></td>
<td>Dielectric fluids in transformers, capacitors, coolants</td>
</tr>
<tr>
<td>BDE-x</td>
<td>Polybromodiphenylether</td>
<td>BDE-28, 47, 99, 153</td>
<td>2009</td>
<td></td>
<td>Flame retardants</td>
</tr>
<tr>
<td>HBB</td>
<td>Polybrominated biphenyls</td>
<td></td>
<td>2009</td>
<td></td>
<td>Flame retardants, see polybromodiphenylether</td>
</tr>
<tr>
<td>HxCD</td>
<td>Polychlorinated dibenzo-p-dioxins (PCDD) (Dioxins)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>PVC production, industrial bleaching, incineration</td>
</tr>
<tr>
<td>PFOS</td>
<td>Perfluorooctane sulfonic acid and its salts</td>
<td></td>
<td>2009</td>
<td></td>
<td>(Fluoro)Surfactant</td>
</tr>
</tbody>
</table>

**PAHs**

<table>
<thead>
<tr>
<th>Name/Synonym</th>
<th>EU, US EPA</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo(a)anthracene</td>
<td></td>
<td>Seven EU PAHs are non-volatile and the most toxic</td>
</tr>
<tr>
<td>Benzo(j)fluoranthene</td>
<td>EU</td>
<td></td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>EU, POPs Protocol indicator, US EPA</td>
<td></td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>EU, POPs Protocol indicator, US EPA</td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>EU, POPs Protocol indicator, US EPA</td>
<td></td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracene</td>
<td>EU</td>
<td></td>
</tr>
<tr>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>EU, POPs Protocol indicator, US EPA</td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Chrysene</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Dibenzo(a,h)anthracen</td>
<td>US EPA</td>
<td></td>
</tr>
<tr>
<td>Benzo(g,h,i)perylene</td>
<td>US EPA</td>
<td></td>
</tr>
</tbody>
</table>
Annex 5

Sampling and analysis of mosses for microplastics

Microplastics are ubiquitous in the environment, as such, their sampling and analysis requires rigorous quality control to avoid contamination (Koelmans et al. 2019; Wesch et al., 2017). Several studies have identified microfibres as the dominant microplastic in atmospheric deposition (Dris et al., 2016; Cai et al., 2017; Allen et al., 2019; Stanton et al., 2019); however, this is likely because microfibres are more easily visually identified. It is difficult to unambiguously visually-discriminate all potential microplastic particles; error rates of visual sorting are reported to range from 20% (Eriksen et al., 2013) to 70% (Hidalgo-Ruz et al., 2012) and increase with decreasing particle size. Furthermore, it is recognised that only spectroscopic analysis (Fourier Transform infrared or Raman spectroscopy) can provide unambiguous proof of the synthetic nature of microplastics (Shim et al., 2015).

A guide to field sampling, laboratory analysis and quality control is given below, specifically focusing on visual identification of microfibres. However, methodologies are rapidly changing given the growing interest in microplastics, as such it is recommended that you review current literature prior to analysis. Nonetheless, all methods employ similar steps, i.e., extraction of microplastics from moss (using wet digestion; Masura et al., 2015) and identification by visual or chemical techniques (Shim et al., 2015). Density separation and dyeing (in case of visual analysis) may also be included in the procedure to help isolate and identify microplastics.

Field sampling. Moss samples should be collected following the Moss Survey manual, wearing nitrile gloves when sampling mosses. It is recommended that 100% cotton clothes are worn during sample collection. The samples (~ 5 g wet weight) should be stored in glass jars that were triple rinsed with filtered B-pure™ water prior to sampling. In addition, a blank sample should be taken at each site to determine contamination during sampling and further processing of the samples. Ideally, several individual samples per site should be taken to determine the variation between samples (but this might not be feasible due to funding limitations, in which case subsamples per site could be pooled as described for elemental analyses).

As mentioned above, methodologies are rapidly changing and therefore it is recommended that you review current literature prior to analysis. Below we provide an example of a method for determining microfibers visually that partly might be applicable to other microplastics too.

Microfibre extractions. In the laboratory, moss samples should be dried (40°C for 48 hrs), weighed (to allow estimation of the number of microfibres per g) and digested using a wet peroxide oxidation method (Herrera et al., 2018; Masura et al., 2015). The digestion procedure is carried out by adding 40 mL of 0.05 M Fe(II) solution to each moss sample; 40 mL of 30% hydrogen peroxide (H₂O₂) is subsequently added and the mixture is left at room temperature for five minutes. The digest is heated to between 40–50°C to increase the reaction, and a further 20 mL aliquot of H₂O₂ added when the reaction slows down (if organic matter is still visible); in general, at least three aliquots will be required. Samples are then vacuum filtered onto glass-fibre filter papers (Fisherbrand™ G6 [09-804-42A]: 1.6 μm) and dyed with 1 mL of Rose Bengal (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein, 200 mg L⁻¹) to help visually distinguish synthetic material from organic matter following Liebezeit and Liebezeit (2014), i.e.,
the non-stained material is typically assumed to be microplastic as Rose Bengal is a biological stain that should bind to natural fibres (Kosuth et al., 2018). Note: the moss sampling jars should also be rinsed with (filtered) B-pure™ water and vacuum filtered onto the same glass-fibre paper to capture residual fibres. The dyed filter papers should be transferred to petri dishes for storage and for assessment of microfibres. You may wish to analysis triplicate 1 g moss samples for each site to assess variation.

**Microscopy and microfibre identification.** Filter papers are visually analysed for the presence of microfibres using a stereomicroscope (e.g., Leica EZ4W with EZ4W0170 camera; ideally a microscope with 40× magnification) following a five criteria visual identification method (Table 1) modified from Norén (2007) and Windsor et al. (2018). Identification of microfibres according to standardized criteria in connection with a strict and conservative examination reduces the possibility of misidentification (Norén, 2007). Further, Löder and Gerdts (2015) demonstrated that for particles > 0.5 mm, visual analyses are suitable for identification. The five criteria are: (i) The fibre is unnaturally coloured (blue, red, green, purple, black, grey) compared with other particles / detritus; (ii) The fibre appears homogenous in material and texture with no visible cell structure or offshoots and is a consistent width throughout its entire length; (iii) The fibre remains intact and is not brittle when compressed, tugged or poked with fine tweezers; (iv) The fibre has a shiny or glossy appearance; and (v) There is limited fraying with no similarities to natural fibres (see Table 1).

**Table 1.** List of criteria used to visually identify plastic microfibres following: (A) four criteria taken from Norén (2007) as cited by Hidalgo-Ruz (2010) and Löder and Gerdts (2015), and (B) eight criteria taken from Windsor et al. (2018), with a recommendation that a positive response for at least two of the eight criteria is required for identification of microplastic particles.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No (cellular) structures of organic origin should be visible in the plastic particle or fibre.</td>
</tr>
<tr>
<td>2</td>
<td>Fibres should be equally thick throughout their entire length and have a three-dimensional bending to exclude a biological origin.</td>
</tr>
<tr>
<td>3</td>
<td>Particles should be clear and homogeneously coloured.</td>
</tr>
<tr>
<td>4</td>
<td>Transparent or whitish particles must be examined under high magnification and with the help of fluorescence microscopy to exclude a biological origin.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unnaturally coloured compared to the majority of other particles/detritus in the sample, e.g., red, bright blue and yellow.</td>
</tr>
<tr>
<td>2</td>
<td>Appears homogenous in material or texture, e.g., no cell structure.</td>
</tr>
<tr>
<td>3</td>
<td>Unnatural shape or structure, e.g. perfectly spherical, smooth or sharp edges.</td>
</tr>
<tr>
<td>4</td>
<td>Fibres that remain intact with a firm tug or poke with fine tweezers.</td>
</tr>
<tr>
<td>5</td>
<td>Shiny or glassy in appearance.</td>
</tr>
<tr>
<td>6</td>
<td>Flexible and can be compressed without being brittle.</td>
</tr>
<tr>
<td>7</td>
<td>Share similar surface characteristics to reference plastic material.</td>
</tr>
<tr>
<td>8</td>
<td>Physical characteristics of fibres are dissimilar to reference natural fibres, e.g., limited fraying.</td>
</tr>
</tbody>
</table>

It is recommended that at least two of the criteria be met for a fibre to be classified as a microplastics (Windsor et al., 2018). Previous studies have classified all fibres not
stained by Rose Bengal as ‘microplastic’ (Liebezeit et al., 2014), while others have chosen to use the more general term ‘anthropogenic debris’ (Kosuth et al., 2018). Spectroscopic analysis (Fourier Transform infrared or Raman spectroscopy) is required to provide unambiguous proof of the synthetic nature of microfibres (Shim et al., 2015). Finally, all microfibres should be photographed and their length measured using image processing software (e.g., ImageJ). Automated routines to count dyed microplastics are also widely used (see Allen et al., 2019).

**Quality control and data analysis.** Throughout sample processing and analysis, procedural open-air blanks should be used to determine the amount of potential contamination; open-air blanks should be exposed during filtering, digesting and oven drying. All B-pure™ water should be filtered (Fisherbrand G6: 1.6 μm) prior to use for cleaning and extraction (use in FE(II) solution and Rose Bengal) to avoid potential contamination. Further, during microfibre extraction (digesting and filtering), all samples should be covered with tin foil to prevent airborne contamination and all equipment should be rinsed with filtered B-pure™ water prior to use. Peroxide blanks (1 L in total) should also be vacuum filtered and analysed following the same method as the moss samples to determine the level of microfibre contamination. Finally, 100% cotton laboratory coats should be worn when extracting and analysing the samples. Where possible the analysis should adhere to quality criteria as identified by Koelmans et al. (2019).

**References**


