National Ecosystems Monitoring Network
Standard Operating Procedure 04
For
Moss Sampling

5-July 2021

Prepared by

University College Dublin (UCD) and the UK Centre for Ecology & Hydrology (UKCEH)

on behalf of the Environmental Protection Agency (EPA)

Citation

Hayes, F., Jones, L.J., Kelleghan, D., Marcham, L., Cummins, T., Rowe E and Tang, Y.S. 2021. Standard Operating Procedure for Moss Sampling, Version 1. University College Dublin, UK Centre for Ecology & Hydrology, Environmental Protection Agency. https://nemn.ucd.ie/nemn-sops/

Acknowledgements

The work was funded by Ireland's Environmental Protection Agency. Thanks to reviewers whose comments helped improve this document: Maria Long of National Parks and Wildlife Service; Ian Marnane of Environmental Protection Agency; Don Monteith and Simon Smart of UK Centre for Ecology & Hydrology.

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1 Scope

1.1 National Emission reduction Commitments Directive, Article 9

EU Member States have committed to reducing emissions of air pollutants to meet targets set in Annex II to the National Emission reduction Commitments Directive (NEC Directive (EU) 2016/2284). Article 9 of the NEC Directive requires each country to develop a network of sites to monitor negative impacts on ecosystems of a group of five important air pollutants (SO_2, NO_X, NMVOC, NH_3 and $PM_{2,5}$) that contributes to acidification, eutrophication, ozone damage and biodiversity loss. The network should be representative of habitat types in the country, with coverage across pollution gradients and co-ordinate with other monitoring schemes for efficiency. Member states report monitoring data to the EU every four years, as required under Article 10 of the Directive in a standard template. Annex V of the Directive also sets out a list of optional parameters the Member States may use in implementing the monitoring and reporting obligation. Vegetation indicators include foliar N content.

1.2 National Ecosystems Monitoring Network (NEMN)

A National Ecosystems Monitoring Network (NEMN) is being developed in Ireland to monitor and report negative impacts of air pollution on ecosystems (acidification, eutrophication, ozone damage and biodiversity loss) under Articles 9 and 10 of the Directive (Kelleghan et al., 2021). Reporting is on four-yearly cycles, with the next reporting of monitoring sites and indicators in 2022 and data in 2023.

NEMN monitoring consists of a set of periodic and continuous surveys, listed in **Error! Reference source not found.**.

In the first reporting round (2018/2019), NEMN included forests (37) and lakes (4), but Annex I open terrestrial habitats were under-represented, with only two sites. In this next phase of the NEMN, sites from five open habitats of critical importance for nature conservation in Ireland have been selected for inclusion. Annex I habitats included are: 6210 calcareous grassland, 6410 *Molinia* meadow, 7110 raised bog, 7130 blanket bog and 4010 wet heath. Sites are located across a gradient of air pollution pressure. Ammonia concentration was chosen as the indicator of air pollution pressure, since ammonia is a direct stressor and contributes to nitrogen deposition.

- All sites will be monitored for air pollution impacts.
- At a subset of these sites, equipment will also be installed to monitor air pollution inputs as well.

Table 1: NEMN surveys, frequencies at Level I and Level II sites, and next report dates.

Survey	Level I sites	Level II sites	Report due
Plot placement	At plot placement		1-July 2022
Vegetation	4 years	4 years	1-July 2023
Soil sampling and analysis	4 years	4 years	1-July 2023
Moss sampling and analysis	4 years	4 years	1-July 2023

In 2021 initial plot placements and surveys are only being conducted in Level I sites of selected open terrestrial habitats. Reporting of site locations and indicators is due 1-July 2022 and 4-yearly, while reporting of monitoring data follows one year later.

1.3 Standard Operating Procedure (SOP) for Moss Sampling and Analysis

Moss sampling and analysis is a survey of the National Ecosystems Monitoring Network. This document sets out details of the method for moss sampling (SOP04) which operates alongside the SOPs for plot placement (SOP01), vegetation survey (SOP02), and soil sampling (SOP03). A further SOP on data management may be added later as this is not fully covered in the current documents. Feedback is invited to improve the SOP at https://nemn.ucd.ie/home/contact/

Monitoring in 2021 will focus on nitrogen deposition, as this is currently the most concerning air pollution type for ecosystems in Ireland, but other pollution types may be introduced later. This draft (June 2021) is for use with the 2021 summer surveys of terrestrial habitats, and is subject to review. The protocol is valid for all terrestrial habitats including forests/woodlands.

Each observational plot at NEMN sites in open terrestrial habitats is based on a permanent quadrat (SOP 01). Data from analysis of moss samples from these locations can be used to investigate effects of air pollution on ecosystems. Carpet-forming mosses obtain trace elements and nutrients direct from the atmosphere (Tyler, 1970). The total nitrogen concentration in mosses can be used as a surrogate to estimate total nitrogen deposition, as mosses show increased nitrogen content with increasing nitrogen deposition, even at the lower ranges of nitrogen deposition (Harmens et al., 2011). Similarly, mosses

provide an approach to assessing atmospheric heavy metal and microplastic deposition to ecosystems (Roblin and Aherne, 2020).

Collection of moss samples and subsequent chemical analysis allows quantification of the air pollution deposition to the vegetation in a simple, standardised way. Concentrations of pollutants (e.g. N) in moss tissue have been widely used to monitor air pollution pressures, which puts data on impacts in context. Analysis for nitrogen content is simplest and requires the smallest volume of moss, whilst analysis of additional moss material for metals content also allows determination of phosphorus content. Phosphorus limitation is an important mechanism governing ecosystem responses particularly when nitrogen deposition is high. Therefore, N:P ratio of moss tissue is a useful metric to assess potential impacts on plant ecosystems.

1.4 Compatibility with other surveys

This SOP is based on that of the International Cooperative Programme on Effects of Air Pollution on Vegetation under the Convention on Long-Range Transboundary Air Pollution (ICP Vegetation) Moss Survey Protocol, and it has been adapted for use within the NEMN habitat surveys. Thus in addition to providing information of direct relevance to the NEMN core requirements, requirements for optional analyses to allow contribution to the ICP Vegetation programme are also included.

2 Objectives

The moss sampling aims to characterise the air pollution environment of each permanent plot. Sampling is therefore required to be representative of the pollution environment, rather than the specific vegetation surveyed in the quadrat.

By taking samples at each of 5 plots per site, variability of air pollution within the site will be measured, and samples remain directly co-located with the other surveys. Replication at the site level helps to account for variation in pollutant signals at the site.

On a national level, the NEMN sites are selected to cover a gradient of nitrogen deposition levels which provides a powerful framework for assessing spatial controls of pollutant pressures on a range of sensitive habitats.

In this survey, samples of single pleurocarpous moss species will be collected for the measurement of nitrogen levels in moss tissue, with the option to conduct analysis for other air pollutants.

3 Positioning

3.1 Sampling design at site level

SOP01 details sampling design at survey and site level for open terrestrial habitats. A total of 375 permanent plots in open terrestrial habitats (Figure 1) are specified for the survey and moss samples will be taken from each one. At forest habitats there will be one permanent plot per site, with a total of 35 permanent plots. Moss samples will be taken from each plot.

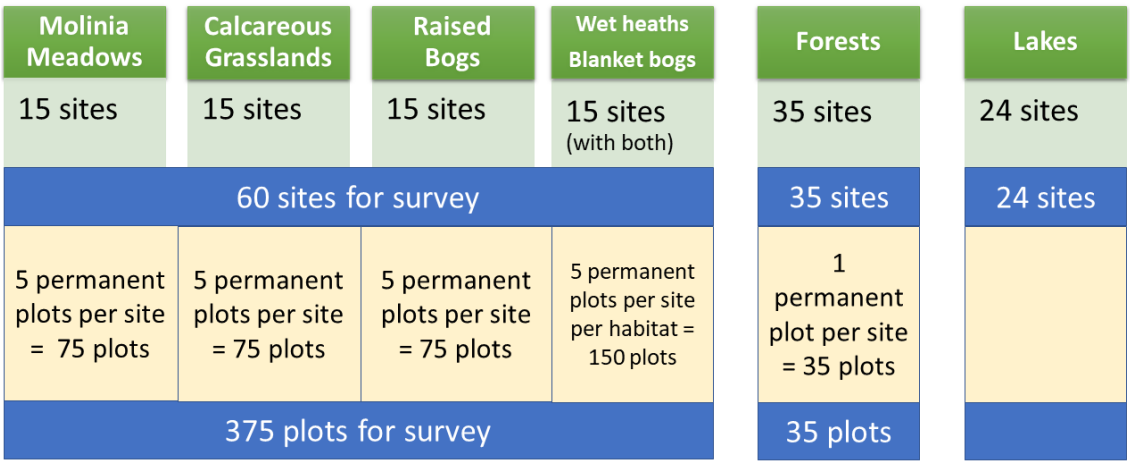

Figure 1. Number of sites and plots for each habitat type in the NEMN. This SOP is not relevant to Lakes monitoring.

3.2 Combining moss monitoring with floristic and soil surveys

Permanent plots will be set up as described in SOP01, so that floristic survey (SOP02), soil sampling (SOP03) and moss sampling (SOP04, this document) are all co-located at the plot level. This means the moss sampling contributes information on the ambient air quality environment the soils and vegetation are exposed to. Moss surveys are undertaken in a zone 2 - 50m around the floristic survey quadrat (Figure 2Figure 2).

3.3 Location and number

If possible, one moss sample should be collected for each plot. As the moss samples represent the pollution environment (rather than the specific vegetation of the quadrat), samples can be taken from different vegetation growing nearby where moss may be more prevalent and should ideally be within 50 metres of the quadrat. If there is no suitable moss present within approximately 50 metres of the quadrat, then no sample can be taken. The sample should be a composite of several sub-samples of a single moss species in order to generate the required volume. The location (GPS coordinates in ITM) of the composite sample should be recorded at the centroid of all the locations of sub-samples. Details of suitable moss species are given in section 4.2.1.

Plot layout

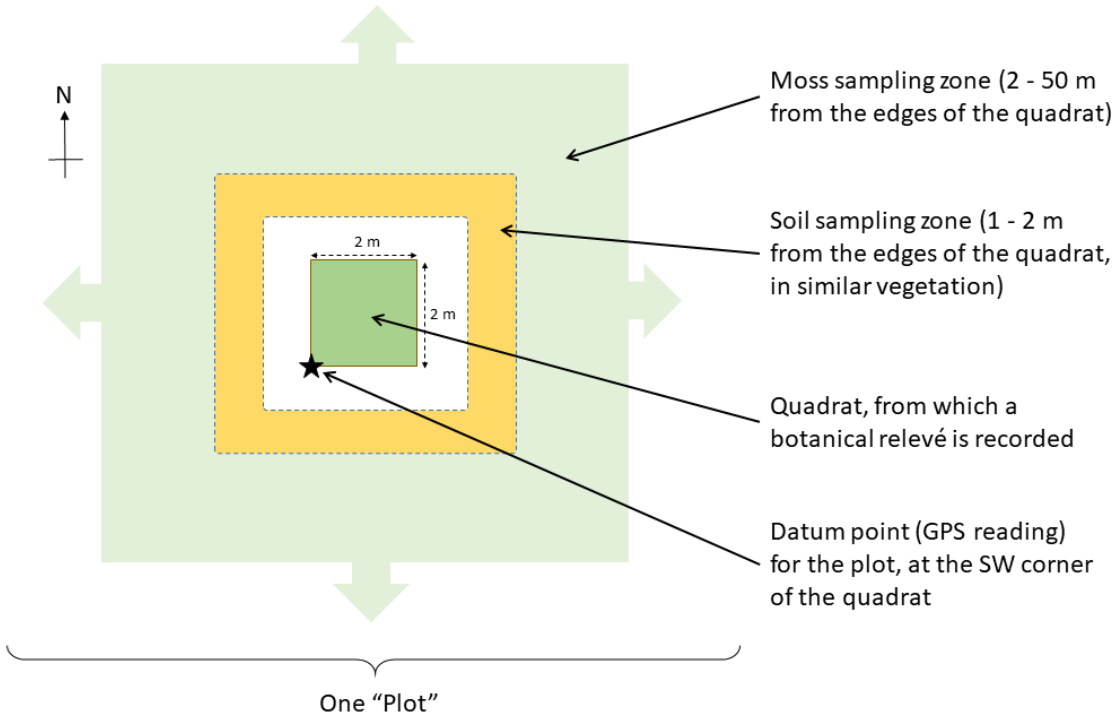

Figure 2. Plot layout. Moss sampling occurs in a zone concentric with the quadrat, between approximately 2 m and 50 m away. Where 4 x 4 m quadrats are used, a similar layout can be used. Please note that moss sampling does not need to be in similar vegetation.

3.4 Sample size for foliar N

A relatively small sample equivalent to a minimum of 0.3 g dry weight is required for C:N analysis. A visual indication of the required amount is shown on the right hand side in Figure 3. It is advisable to collect between 3 – 4 g of moss (fresh weight) to ensure there is sufficient sample for analysis after processing (discarding old growth, etc.) and drying.

Figure 3. Minimum volume of moss required for C:N analysis. On the right is 0.3 g dry weight 'new, green' moss for analysis, on the left is a discarded 'old growth' portion of the total sample. Note that after air drying or drying at low temperature the moss remains green.

For 2021, only the above collection for N is needed. Methods below for metals, P and microplastics will not be applied in 2021, but are included for information as they are likely to be needed in future surveys.

3.5 Sample size for other parameters

For analysis of metals and P (which allows determination of N:P), a litre of a single species of moss is required.

Analysis for microplastics requires an additional litre of a single species of moss (although this can be a different moss species to that for metals, P and N analysis, if necessary.

Note that these are not proposed for 2021.

3.6 Markers

The location of the moss sample does not need to be marked at the site. However, the location should be recorded using GPS, as the centroid of the sub-samples.

3.7 Additional information

Only pleurocarpous mosses should be sampled. The preference is *Hylocomium splendens* or *Pleurozium schreberi*. If it is necessary to use others, the first choice is *Hypnum cupressiforme*, followed by *Pseudoscleropodium purum*, then any other pleurocarpous moss. A brief guide to these mosses is given in section 4.2.1. It is important to identify the species of moss correctly, as different moss species can respond more strongly to atmospheric nitrogen deposition than others.

4 Sampling

4.1 Type of Measurements and reporting units

The location of the composite sample (as the centroid of the sub-samples) and the species of moss collected should be recorded on site, together with some additional parameters about the collection site that are useful for subsequent data analysis and interpretation (detailed in section 4.4). Although the sub-samples will likely be collected over a relatively small area, the distance and direction from the quadrat will not be uniform for all plots. It is easiest, but not essential, if the same moss species is collected at the site in subsequent sampling rounds. Similarly, it is ideal but not essential to collect moss from a similar area in subsequent sampling rounds.

4.2 Field Method

Surveyors need paper bags for the samples with pre-printed labels, a mechanism for recording of the plot data (either electronic or on paper), site maps and a GPS unit. If gloves are used, nitrile, cotton or silk gloves should be worn, not latex or any powdered gloves as they can contaminate the samples. For microplastics sampling, the surveyor should be wearing clothes which do not contain any plastics as clothes shed microplastics onto the samples. If this is not possible, then sleeves should be pulled up to the elbow, and leaning over the sampling area and brushing hands against clothing should be avoided.

The moss survey zone of the plot should be searched for *Hylocomium splendens* and *Pleurozium schreberi.* If neither of these is present search for *Hypnum cupressiforme*, followed by *Pseudoscleropodium purum.* If these species are also not found, search for any other pleurocarpous moss species. Deposition is greater under trees and shrubs, as rainwater collects more pollutants from the surface area of the tree as it drips through the canopy. Thus, it is preferable to avoid areas within 10m of a tree, but where sampling is closer, the distance from the tree is recorded. Running water should be avoided too. Moss sampled within 100m of minor roads or 300m of major roads in rural areas should not be submitted to the ICP Vegetation Moss Survey.

Once a species is found in sufficient quantity, the moss should be picked, preferably from several sub-sample points within the zone. Suitable locations for finding moss near to the quadrat include ranker grassland next to hedges, on decaying logs and tree stumps, or near to heather. The objective is to represent the air pollution of the plot, therefore, the vegetation where moss is collected does not need to be the same as the vegetation of the quadrat. Pinching a small amount of moss between fingers and thumb and gently pulling is a good method to extract moss from an area of mixed and intermingled vegetation. Avoid sandy moss or any with ants in it. Litter and animals should be removed from the moss sampled. The label for the bag should be completed then the moss placed in a paper bag. After the moss has been sealed into a paper bag (e.g. by folding over the open end several times), the paper bag can go into a plastic bag if necessary (e.g. if it is raining, or for transport).

4.2.1 Brief guide to the main mosses

Hylocomium splendens: Stems regularly bipinnate. Shoots can be 10 – 20 cm long. Upper part of leaves sharply toothed. Commonly found amongst grass and heather and in acidic woodlands.

Pleurozium schreberi: Red stems, loose growth pattern, pinnate. Shoots several cm long. Leaves 2 - 2.5 mm long with a blunt tip. Commonly found amongst grass and heather.

Hypnum cupressiforme: Irregularly branched, shoots approximately 20 mm long, with curved leaves 1 - 2 mm long that taper to a long, fine point. The leaves are untoothed and overlap. The nerve is absent or very short. Can grow on decaying wood.

Pseudoscleropodium purum: Pinnate shoots that can be >10 cm long. Leaves only slightly longer than wide, so can appear quite 'robust'/'stout'. Small recurved point at the end of the leaf tip. Often found in unimproved grassland, including calcareous.

Downloadable pdf pages with photographs and more on identification are available from: https://www.britishbryologicalsociety.org.uk/learning/species-finder/

4.3 Sample handling

The paper sample bags need to be labelled so they are uniquely identifiable. The label should include:

- Site number
- Plot number
- Date (yyyymmdd)
- Analysis

Together, these make up a unique sample ID. Moss species is suggested for inclusion on the label but will also be recorded in the data form so is not essential if label space is a problem.

Pre-printed labels will ensure all data fields are completed. A template label is provided in Appendix I. Bags should not be squashed in transport (in case they burst, especially if they are damp) but do not need to be kept cool.

Unless the samples are going to the laboratory within two days, they should be air-dried at room temperature. The paper bags can simply be spread out in a warm ventilated place until dry, perhaps turned to ensure drying all around. Once dry, they should be stored in a dry, dark place. Contractors are responsible for this, along with arrangements for storage of samples until the laboratory analysis.

4.4 Data recording

Data about the sample and location are recorded in the field. The list of parameters to record for each sample, includes:

- Moss species
- Number of sub-samples (1 - 5)
- Whether mosses sampled occurred sparsely or in cushions or mats
- Broad vegetation of moss collection (e.g. hedge, rough grassland, woodland fringe, heath)
- Approximate distance from quadrat
- Slope or flat
- Distance to nearest tree canopy (0 m if under a tree, up to >10 if none)
- Intended analysis (relevant to future surveys when several analyses are included)

A google form is available to record this data at https://nemn.ucd.ie/documentation as plot_moss If no suitable mosses are found in the plot, a record should be made of this by listing moss species as 'none'.

In future, if samples are required for the measurement of parameters in addition to N, it will be necessary to collect more than one sample per plot.

4.5 Laboratory analysis method

Carefully clean all dead material and attached litter so that only green shoots from last 2 – 3 years growth are included. Samples for analysis should be dried to constant weight at 40°C.

Dried samples should be milled to a fine dust prior to analysis. Keep a record of the analytical procedures used. Units of concentration must be given, together with the detection limit of the applied technique.

4.6 Quality assurance and quality control

Standard samples of known concentrations should be included in sample analysis runs for quality assurance purposes.

5 Data Handling

5.1 Data capture

Surveyors may use the moss_plot Google form at https://nemn.ucd.ie/documentation to enter plot data while in the field. A spreadsheet with the headings should also be carried in case of there being no internet signal to fill out the google form. Responses to the Google form go into a spreadsheet which can be saved in .csv format for processing and submission.

5.2 Data validation

- Check that the moss species recorded is 'feasible'.
- Use standards in chemical analysis.
- The range of likely content of N and the various metals in the sample is given in
- Table 2, using the results of the 2015/2016 moss survey.

Table 2: Metal (mg kg^{-1}) and nitrogen concentrations (mass %) in mosses in Ireland in 2015/16 (Frontasyeva et al., 2020).

	As	Cd	Cr	Cu	Fe	Hg	Ni	Pb	V	Zn	Al	Sb	N (%)
Ireland													
Number	127	108	109	131	131	130	61	131	131	131	131	130	131
Min	0.0002	0.0002	0.037	1.34	42.0	0.010	0.050	0.14	0.14	4.86	22.4	0.010	0.45
Max	0.97	0.43	5.31	47.7	874	0.074	7.42	65.8	2.01	167	815	0.44	1.33
Mean	0.12	0.071	0.76	4.30	148	0.036	1.30	2.18	0.56	30.6	133	0.065	0.78
Median	0.082	0.049	0.46	3.23	107	0.034	0.64	0.74	0.49	22.3	100	0.048	0.74
90th percentile	0.19	0.17	1.68	5.92	228	0.052	3.61	2.31	0.99	59.6	223	0.13	1.04

5.3 Data processing

A further SOP on data management will be prepared later as it is not fully covered in these SOPs. Data from the contractors to the EPA should be submitted as .csv files.

6 References

Frontasyeva M., Harmens H., Uzhinskiy A., Chaligava, O. and participants of the moss survey (2020). Mosses as biomonitors of air pollution: 2015/2016 survey on heavy metals, nitrogen and POPs in Europe and beyond. Report of the ICP Vegetation Moss Survey Coordination Centre, Joint Institute for Nuclear Research, Dubna, Russian Federation, 136 pp. ISBN 978-5-9530-0508-1.

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Roblin, B. and Aherne, J. (2020) Moss as a biomonitor for the atmospheric deposition of anthropogenic microfibres. Science of the Total Environment 715:136973.

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7 Annex I

7.1 Template moss sample label

<table>
<tr><td colspan="4">National Ecosystems Monitoring Network</td></tr>
<tr><td colspan="4">Moss Sample: Air dry within 2 days of sample collection, then store in dark, dry conditions.</td></tr>
<tr><td>Site

N000</td><td>Plot

00</td><td>Date

yyyymmdd</td><td>Intended analysis

Nitrogen</td></tr>
<tr><td colspan="3">Moss species (tick one)
○ Hylocomium splendens
○ Pleurozium schreberi
○ Hypnum cupressiforme
○ Pseudoscleropodium purum
○ Other (specify):</td><td>Comments: (optional)</td></tr>
<tr><td>NEMN Moss contact</td><td colspan="3">Felicity Hayes: fhay@ceh.ac.uk</td></tr>
</table>