

Review

Use of Moss Bags as Active Biomonitors for Ambient Air Quality—Practices, Variability, and the Current State of Knowledge: A *Scoping Review* and Call for Future Research

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Abstract

Active biomonitoring using moss bags provides a cost-effective alternative to traditional sensor-based air quality networks, directly supporting multiple UN Sustainable Development Goals, including SDGs 3, 7, 11, and 13. However, the reliability of moss bag outputs is frequently challenged due to the wide-ranging variability in moss bag construction and exposure. To critically evaluate these disparities, this scoping review employed the PRISMA framework to synthesize 137 moss bag studies between 2005 and 2025 to assess the dominant moss bag parameter choices. The results revealed a ubiquitous lack of a “standardized operating protocol (SOP)” in almost every aspect of moss bag studies, which restricts the replicability of experiments. The preparatory stages revealed widespread “customization” of moss sampling methods; the use of water as the main moss cleaning–washing medium (~87% of the literature reviewed herein); drying at 105 °C (~80.7%); and a general preference for the *Sphagnum* species (74%). Regarding the constructional phases, preferences for cylindrical bag shapes (60% of the literature reviewed herein) and nylon mesh for the bag material (68%) were noted. However, the mesh aperture sizes (<1 to >10 mm²) and bag exposure duration (<4 to >10 weeks) exhibited ranges far too broad for cross-study replication. Less than 10% of the literature explicitly mentioned choices of moss packing density, bag elevation, irrigation, shading, and orientation. Reflections on the influences of environmental/meteorological variables on moss bag results were sparse. Less than 5.5% of the literature delved into any process-level discourses for parameter choices, while barely 2.8% of the literature attempted to establish SOPs for moss bags. Overall, this study highlights that much remains to be learned about the moss–pollutant interaction pathways, phytotoxicity, and chlorophyll damage, and that moss bag studies still need to be streamlined; they still lack clarity in their reporting of study designs/parameterization choices, which could collectively limit the future use of moss bags to support air quality policy and decision-making.



Academic Editor: Yanling Deng

Received: 6 July 2026

Revised: 7 August 2026

Accepted: 19 August 2026

Published: 27 August 2026

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Keywords: air quality; moss bag biomonitoring; constructional variability; standard operating procedures; moss phytotoxicity; chlorophyll status; Sustainable Development Goals (SDGs)

1. Introduction

Recent global projections indicate increased air quality degradation with a concomitant rise in related public health hazards in the coming years [1]. The latter is expected to escalate further on a warming planet [2] due to the accumulation of a wide array of

atmospheric aerosols and a variety of greenhouse gases [3,4], undermining global sustainable development agendas. This marks an urgent need to ramp up current air quality monitoring capacity and efficiency to support process-level research, leading to more data-driven interventions for air pollution control and/or mitigation [3,5,6]. The need for the latter has accentuated further in the post-SARS-CoV-2 periods [7,8], as air quality-related health hazards have become increasingly apparent worldwide, affecting the health and well-being (UN SDG 3) of a vast number of people. A great wealth of research reports on the health impacts of elevated concentrations of particulate matter [9,10] and heavy metals [11,12] in ambient air. Orellano et al. [13] conducted a systematic meta-analysis of 106 global cohort studies commissioned for the World Health Organization (WHO), finding high-certainty concentration–response evidence linking long-term exposure to PM_{2.5} and PM₁₀ with elevated all-cause, cardiovascular, and respiratory mortality. Ma et al. [14] assessed the multi-year seasonal variations of 11 airborne trace elements (e.g., Pb, Mn, Cr, As, Sb, Cd, Ni) in fine particulate matter, showing increased multi-pathway toxicity (inhalation and dermal absorption) and identifying elevated cancer risks ($>10^{-6}$) from As and Cr, specifically among children.

1.1. Moss Bag Literature Survey

1.1.1. Why Moss for Biomonitoring?

In view of the surging risks of air quality degradation worldwide, a growing body of research explores potential opportunities to strategically use various biomonitors to assess air quality (and changes therein). A large body of literature characterizes naturally occurring biomonitors, which are cheaper than traditional machine-based automatic instruments, and have a demonstrated ability to account for airborne pollutant concentrations. The most popular biomonitors have included various tree species [15,16], lichens [17], and mosses [18–22]. Moss bags have proven more effective than the others [23]. For example, moss bags (*Sphagnum capillifolium* and *Hypnum cupressiforme*) were found to have a greater capacity to intercept trace elements and polycyclic aromatic hydrocarbons (PAHs) compared to lichen (*Pseudevernia furfuracea* and *Parmelia sulcata*) and did so more efficiently [24]. Coskun et al. [25] also found mosses to be more effective in the biomonitoring of metals than lichens. In a study in Wuhan city, China, Jiang et al. [26] compared a moss species (*Haplocladium angustifolium*) with two evergreen tree species (*Cinnamomum bodinieri* and *Osmanthus fragrans*) and found that the moss had a superior air quality monitoring capacity.

Moss bags allow the monitoring of long-term pollutant levels, as compared to the daily/hourly measurements of traditional air quality instruments (which thus lack long-term integrative capacity) [27]. Additional challenges of such traditional instruments include high costs (for installation, repair, and maintenance) and a lack of adequate spatial representation [3,5]—traditional sensor networks can only be installed at selected spots, limiting their spatial distribution. Mosses, being naturally growing species, can be distributed more widely, offering more “representative” spatial samples. Moreover, as mosses lack cuticle layers and real root systems, the nutrients and/or pollutants found in their tissues are obtained directly from the atmosphere, making their levels representative [26]. In addition, the mosses’ morphologies do not vary with the seasons, making them low-cost biomonitors for air quality year-round [28]. Unlike traditional sensors, moss bags also provide estimates of multiple air pollutants simultaneously (inorganic and organic) [22,29,30].

1.1.2. Moss Bag Applications

Moss-based biomonitoring takes two principal forms: passive and active [31]. While the former deploys natively occurring mosses [32,33], the latter uses transplanted moss placed within specially designed bags (of various dimensions and materials) exposed to

ambient atmosphere for various times to intercept pollutants [19,34]. In urban habitats, poor air quality has particularly become a threat to human development and environmental sustainability and protection [35–38], threatening the core foundations of urban sustainable design (UN SDG 11: building resilient cities and communities).

Moss has seen a wide variety of applications ranging from land use and land-cover changes [39,40], to indoor–outdoor air quality differentiation [41,42], to detecting seasonality in air quality [43,44], to pollutant source detection and apportionment [45], and even to the assessment of particulate matter concentrations following volcanic depositions [34,46]. What makes moss bags ideal for air quality monitoring is their ability to respond to a wide array of pollutant types, including heavy and trace metals [26,31,45], metalloids [46], atmospheric radionuclide species [47], microplastics [48], methylated arsine emissions [49], dust [50], and polycyclic aromatic hydrocarbons (PAHs) [22,51–53].

Over the years, moss bags have become potent biomonitoring tools for diverse air quality assessments in urban ecosystems [48,49,52,54]. For example, Hu et al. [55] used *Sphagnum junghuhnianum* moss bags to monitor the traffic volumes in Wuxi City, China (Jiangsu Province). The study identified elevated levels of chromium (Cr), copper (Cu), lead (Pb), vanadium (V), and zinc (Zn) in urban air. Computing the relative accumulation factor (RAF) and contamination factor (CF), the authors demonstrated the effectiveness of moss bags as biomonitors to distinguish seasonal differences in pollutant levels (e.g., higher environmental health threats due to elevated heavy metal concentrations in the winter season than in summer). In another study, Limo et al. [56] used the magnetic characteristics of a series of metals (e.g., Cu, Mo, Sb) using moss bags (*Sphagnum papillosum*) to study point source emissions associated with stop-and-go traffic in the city of Turku, Finland. Their study revealed the effectiveness of the moss bags by identifying the environmental health threats associated with stop-and-go traffic patterns (e.g., at traffic light crossings vs. mid-way areas) commonly found in almost all urban hubs around the world [57]. Moss bags have also been used in evaluating urban road dust distribution and composition [45]. Overall, moss bags have become active instruments in environmental biomonitoring initiatives, helping urban sustainability planners and decision-makers with data-driven policy drafting.

1.1.3. Moss Bag Research: Geographic Distribution

Ambient air quality monitoring using moss bags has recently been documented in several countries, including Finland [43–45], Belgium [58,59], Bulgaria [60], Italy [39–41,61], Germany [62], Serbia [38], Kosovo [63], Poland [64], Albania [18,65,66], Iran [67], Greece [68], India [69,70], and China [71]. The most wide-ranging experiments on moss bags were part of a 5-year European transnational joint monitoring program that included over 100 researchers across 28 countries [28].

The pronounced geographic asymmetry in moss bag biomonitoring literature—overwhelmingly dominated by European studies—is probably driven by structural governance, established research infrastructure, and eco-physiological factors. For example, in the European nations, long-standing coordination under the UNECE (United Nations Economic Commission for Europe) ICP Vegetation (International Cooperative Program on Effects of Air Pollution on Natural Vegetation and Crops) framework has provided standardized sampling protocols, cross-border funding, and systematic regional surveys that have significantly lowered the entry barriers for active moss bag studies [28,29].

Conversely, the relatively lower/sparse moss bag adoption across North America stems from a stronger institutional reliance on automated regulatory physical sensor networks (e.g., US EPA Federal Reference Methods), prioritizing continuous instrumental monitoring over biologically integrated exposure metrics [39,40].

In the more tropical and arid developing regions across the Global South, widespread implementation remains constrained by the scarcity of native, high-cation exchange capacity (CEC) *Sphagnum* species, rapid physiological desiccation during extreme heat exposure, and limited local taxonomic infrastructure [72].

Consequently, a critical monitoring paradox emerges: rapidly urbanizing centers experiencing severe ambient pollution loads remain the least equipped to leverage active biomonitoring as a scalable, low-cost alternative to capital-intensive physical monitoring stations.

1.2. Study Hypothesis and Objective

The main hypothesis of the present study was as follows: the methodological divergence observed in the moss bag literature is marked by contextual customization, rather than compliance with any universally standardized protocol, resulting in systematic reporting gaps across studies.

To that end, this study aimed to summarize the observations from related literature spanning the past two decades (2005–2025) to highlight the variability in moss bag practices [29,31,32,41]. This ensues from the observation that, despite the growing number of applications in various fields, moss bag results continue to be questioned [30,31,73,74] due to widely differing parameter choices, ranging from moss handling routines and bag construction to exposure routines, due to a lack of clarity in reporting regarding the exact parameterization of study designs [50,75,76]. Such variability not only jeopardizes the reliability of moss bag results (e.g., the concentrations and distributions of various air pollutants), but also makes comparison across moss bag studies difficult [77].

The current study presents a scoping review of two decades (2005–2025) of moss bag literature, using the PRISMA 2020 (Preferred Reporting Items for Systematic Reviews and Meta-analyses) technique. The driving idea was that such a ‘scoping review’ could lay the future foundations [78,79] of a more detailed ‘systematic review’ of the same [80] to prompt future researchers to be clearer in their exact parameter choices and reporting.

1.3. Potential Study Novelty

A prime contribution of the present study to the advancement of the relevant research is that the previous literature has mostly ‘cataloged’ regional deployment data, yet a definitive evaluation of the processing of the ‘inconsistencies’ has remained largely absent [81]. The present study addresses this ‘gap’ in the moss bag literature by offering future researchers a roadmap for standardizing moss bag usage. Moreover, unlike a purely bibliometric analysis, this scoping review presents a literature review-based mechanistic evaluation, exploring how specific practices tend to influence moss–pollutant interactions and, thus, the overall results.

To that end, the study also underscores the urgency of establishing a more ‘universally accepted standardized operating protocol (SOP)’ that more comprehensively defines methodological approaches, making moss bag results (e.g., ambient pollutant concentrations) comparable across studies. This is critical, as Salo and Makinen [82] observed: “Since the moss bag technique has no international standard, the results from different studies or countries may be difficult to compare” [83]. In the process, the present study also reflects on a sparse body of research that has undertaken such initiatives and recommends specific values and/or ranges of values for various moss bag parameters.

A more critical contribution is the reflection on less common moss bag parameters, such as moss bag irrigation, orientation, and shading, which are rarely mentioned explicitly in the literature but could be consequential in interpreting moss bag results. Moreover, to highlight the importance of various critical parameters, this study also presents a critical

discourse on the process-level significance of various moss bag parameters, which could help the research community make more informed choices in the future. The final parts of this scoping review also highlight the current knowledge gaps in moss bag research and, by the same token, potential future research directions [73] that could improve moss bag performance, while increasing the reliability and replicability of the results.

Overall, the present study is driven by the idea that the efficient use of moss bags (i.e., standardized choices of parameter values/ranges) could offer an advantage to improve air quality monitoring networks (e.g., more spatially intensive data generation, lower investments than installing sensor-based, instrumental monitoring and troubleshooting) to help establish more informed and data-driven pollution control–prevention interventions [75,84] provided that the variabilities in parameterization are minimized.

2. Methodology

2.1. Overview of PRISMA Approach for Scoping Review

In the first stage, documents were identified using the PRISMA checklist, which comprises 27 elements, including the title, abstract, keywords, and main text [85–87]. The details of the PRISMA procedure are presented in Supplementary Material Table S1. The process comprised five steps, as described below and shown in Figure 1. The literature search was performed in two strands:

- Strand I: Searching the database;
- Strand II: Cross-referencing the literature.

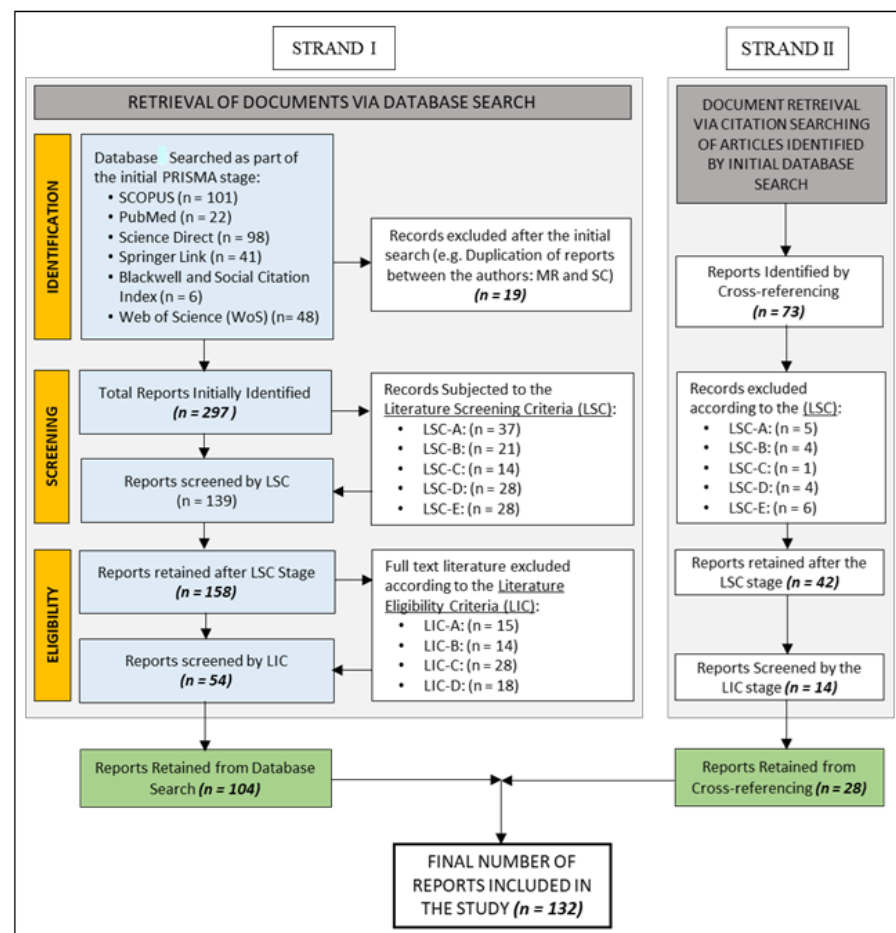


Figure 1. Broad schematic of sequential stages of PRISMA approach: literature search, screening, eligibility test, and final inclusion in the study (NOTE: see the main text for the explanations of LSC and LIC) (authors' own illustration).

The strands were carried out by both authors (MR and SC). Strand II was conducted by carefully scrutinizing the bibliography of the literature identified in ‘Strand I’ to obtain additional material. Each strand comprised several sequential stages of literature screening and testing for eligibility for inclusion in the final text.

2.1.1. PRISMA STAGE I: Identifying the Main Document-Sourcing Databases

Information searches were carried out on online databases with large academic study repositories, namely [16] the following:

- SCOPUS;
- PubMed;
- Science Direct;
- Springer Link;
- Blackwell and Social Citation Index;
- Web of Science (WoS).

2.1.2. STAGE II: Literature Selection

The literature from the above databases was selected through three main steps:

Using pre-determined keywords/phrases relevant to the present study: the search keywords entered for the initial document search included the following [22,24]:

“moss bag”, “active biomonit*”, “metal”, “PAH”, “urban”, “street*”, “moss collection”, “moss cleaning”, “moss washing”, “moss cleaning”, “moss drying”, “Sphagnum*”, “bag material”, bag exposure *, “mesh opening size”, “moss weight”, “moss surface area”, “bag elevation”, “bag shape”.

These search phrases were selected based on the authors’ previous experience exploring the moss literature [32,88], as well as from conducting other literature review-based studies in various other fields of environmental quality management [16,85,86].

Various combinations of search phrases were often employed, using Boolean operators like AND and OR as appropriate to yield the maximum ‘hits’ on the relevant literature in the search databases. While combining them, each search phrase bore the term “moss bag”, as a suffix and/or prefix.

The asterisk symbol (‘*’) was used in various search phrases as a wildcard character to expand the search horizon. For example, the phrase “sample*” included both ‘sample’ and ‘sampling’.

Overall, the initial document search phase comprised the following:

- Exploring the title, abstract, and keywords of the literature found through the above steps in depth.
- Subjecting the literature retained following the above to a rigorous ‘elimination/retention’ procedure by carefully assessing the entire literature.

2.1.3. PRISMA STAGE III: Data Collection Routines

This stage comprised an elaborate (and time-consuming) analysis of the actual content of the literature selected in STAGE II. In this process, all sections of the literature—the title, abstract, methods, research results, conclusion, and references—were critically scrutinized.

2.1.4. PRISMA STAGE IV: Setting Literature Screening Criteria (LSC)

The main impetus was to establish a transparent and logical method of screening a certain ‘kind’ of article—that is, those identified by the initial database search (STAGE II) and finally included in the main text of the current review. The criteria were set as follows [78,89]:

- LSC-A: Duplication of literature (e.g., updated versions of the same study and authors).

- LSC-B: No additional insight other than what has already been found.
- LSC-C: Full text not found.
- LSC-D: Too many confounding variables.
- LSC-E: Lack of much focused discussion.

In addition to the above, any literature identified by the search phrases but falling outside the established time window for this study (2005–2025) was manually eliminated.

2.1.5. PRISMA STAGE V: Setting Eligibility Criteria

Concurrent to setting up the LSC in the previous stage (STAGE IV), the literature inclusion criteria (LIC) were established as follows:

- LIC-A: Any primary and secondary research retained from the previous stage (STAGE II) and written in English.
- LIC-B: Clearly articulated objectives around moss bag results.
- LIC-C: Substantial enumeration of moss bag construction and bag exposure procedures.
- LIC-D: Offering at least some ideas about the study limitations and future needs.

2.1.6. PRISMA STAGE VI: Literature Inclusion

The type of literature finally included in the mainframe of the scoping review ($n = 110$) included peer-reviewed manuscripts, book chapters, technical documents, theses, and dissertations.

Throughout the process, both authors (MR and SC) independently evaluated the initially retrieved literature (identified through various keywords and search phrases) to assess their eligibility for inclusion in the final narrative, based on the information quality (e.g., new findings and/or better explanations of the moss bag parameters). Any disagreements were critically discussed and resolved.

2.2. Study Limitations

While the present narrative offers a scoping review of the literature, it restricts itself to providing a broad overview of current (2005–2025) practices in moss bag parameterization. This study does not delve into a full-scale assessment of the risk of bias of the evidence gathered, which is more commonly expected of a systematic review [90,91]. To that end, this study is a scoping review of the relevant moss bag literature that (1) offers a broad, summary view of the key findings in the recent moss bag literature; (2) revisits the core tenets of the same to expound the theoretical underpinnings of moss-based biomonitoring, its growing applications, and challenges therein; and (3) points to knowledge gaps to point to potential future research directions. To that end, an underlying motto herein is also that it will lead to a systematic review of the literature in the future [92].

3. Results

3.1. Geographical Distribution and Study Contexts

To map the spatial extent and operational environments of active moss bag biomonitoring across the included literature, studies were categorized by global region, country, and exposure setting (Table 1). The extraction reveals a heavy geographic concentration in Southern, Eastern, and Central Europe, which collectively account for over 80% of active biomonitoring applications in the reviewed pool. Italy and Spain represent the most heavily studied nations in Southern Europe, focusing primarily on urban street canyons, petrochemical complexes, and volcanic emissions. Eastern and Southeastern European studies (e.g., those in Serbia, Poland, and Albania) predominantly utilize active transplants to evaluate industrial pollution gradients and urban background quality.

Table 1. Geographical breakdown and environmental contexts of active moss bag biomonitoring studies included in the review. Data source: *authors' own analysis based on the literature included in the present study.*

Region	Countries	% in This Study	Primary Exposure and Environmental Contexts
Southern Europe	Italy, Spain	~42%	Urban street canyons, vehicular traffic, petrochemical/industrial plants, volcanic emissions (Etna, Vulcano), coastal gradients
Eastern and Southeastern Europe	Serbia, Poland, Albania, North Macedonia, Kosovo, Bulgaria, Romania	~26%	Urban background monitoring, industrial complexes, car workshops, indoor vs. outdoor air quality, regional atmospheric deposition
Northern and Central Europe	Finland, Germany, Austria, France, United Kingdom, Czech Republic	~15%	Atmospheric microplastics/microfibers, magnetic monitoring, road tunnel emissions, biosphere reserves, seasonal snow/moss comparisons
East and South Asia	China, India, Japan, Vietnam	~10%	Megacity traffic gradients, industrial mining/bauxite areas, radionuclide interceptors post-Fukushima, toxic metal precipitation
Eurasia and Middle East	Turkey	~4%	Urban bioclimatic planning, tin and heavy metal traffic monitoring
Americas	Canada, Chile, Brazil, Ecuador	~3%	Microplastic deposition, Neotropical Andean urban centers, semi-arid wind/porosity assessments, genetic/bioaccumulation comparisons
Africa and Oceania	—	<1%	Identified as major literature gaps in active moss bag deployment

Outside Europe, active moss bag monitoring is concentrated in East and South Asia (notably China and India), where studies focus on industrial clusters, megacity traffic, and heavy metal deposition. Despite the versatility of the moss bag technique in semi-arid and tropical urban environments, studies from Latin America, North America, and Eurasia (Turkey) remain relatively sparse, while African and Oceanian ecosystems represent significant gaps in active biomonitoring literature.

3.2. Moss Species Selection

Assessment of the recent (2005–2025) moss bag literature revealed a predominance of the *Sphagnum* sp. for moss species (about 81% of the literature reviewed in this study), followed by the *Hypnum* and then by others (e.g., *Pseudoscleropodium* sp., *Brachytecium* sp.) (Figure 2a) [69]. This is probably due to (1) the larger surface area of *Sphagnum*, and (2) the abundance of ion exchange sites, which collectively facilitate pollutant *entrapment* through the formation of complexes [62,93–95]. In a batch experiment involving the *Sphagnum* sp. alongside others (*Hypnum* sp., *Pseudoscleropodium* sp., *Brachytecium* sp.), González et al. [96] found the *Sphagnum* sp. to be the most efficient adsorbents of pollutants due to the presence of a higher number (0.65 mmol/g) of surficial functional groups (e.g., phosphodiester, carboxyl, phosphoryl, amine polyphenols), capable of intercepting a wide variety of pollutant types. Within the *Sphagnum* species, the *S. palustre* variety was dominant, accounting for over 74% of the total *Sphagnum* moss bag literature reviewed herein (Figure 2b).

3.3. Moss Handling—Initial Quality Control Checks

3.3.1. Sampling—Collection and Storage

Ensuring the quality of moss bag results (e.g., the reliability and replicability of outcomes) begins by following established protocols for moss collection from a *pristine* environment. About 63% of the literature reviewed herein acknowledged the importance of the above to ensure that the initial concentrations of the target pollutants were low within the moss tissues [29,97].

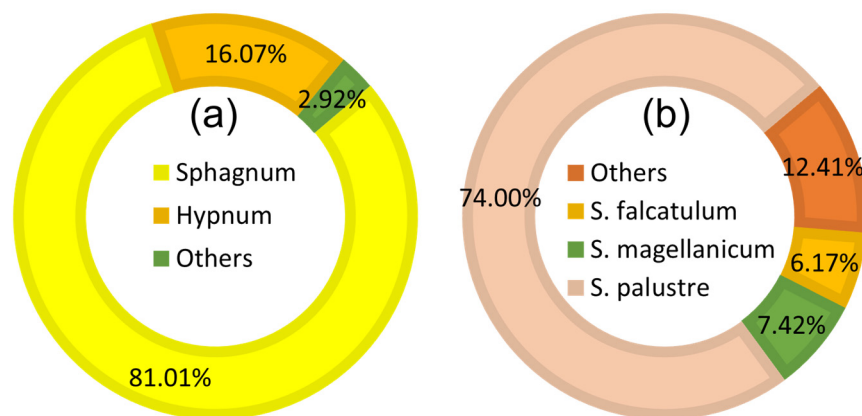


Figure 2. Relative percentages of (a) various moss species and (b) species within the *Sphagnum* variety itself used in the moss bag literature reviewed herein. NOTE: The numbers for each panel represent the relative percentages. (Data source: data compiled from various literature; authors' own illustration.).

In this regard, the ICP-Vegetation (International Cooperative Program on Effects of Air Pollution on Natural Vegetation and Crops) was established in 1987 with the aim of advancing the understanding of damage caused to plants by air pollutants. Presently, over 50 countries are part of the initiative. The ICP-V initiative comes as part of the Convention on Long-range Transboundary Air Pollution (LRTAP Convention), covering the UNECE (United Nations Economic Commission for Europe) region of Europe and North America [98]. Within the ICP-V framework, moss surveys have been conducted on a continental scale in various European countries, every five years since 1990, to assess the ambient levels of a wide variety of metals and persistent organic pollutants (POPs) [99]. The program in [98] outlines a 'standardized' moss sampling rubric (Figure 3). For example, the ICP-V manual emphasizes that collectors should observe the following rules during collection:

- Wear disposable plastic, non-talcum gloves to avoid any type of physical contamination that might introduce bias;
- Avoid smoking because tobacco smoke contains heavy metals and/or organic species that could 'distort' the chemical analysis results.

However, only 24.6% of the literature reviewed herein explicitly detailed the sampling strategy, and only about 11.6% of those studies complied with all the ICP-V recommendations.

Only about 7.3% of the literature reviewed herein either clearly mentioned and/or detailed the exact moss collection specifications, and only about 3.5% of the literature adequately complied with the recommended ICP-V practices. Most of the studies reviewed had opted for some method customization for moss sampling, devising their own strategies based on the specific study context [100]. Though such approaches offer 'convenience', the lack of sampling strategy standardization risks the results becoming 'non-replicable' across various studies in the same region.

The ICP-V recommends two types of practices for storing moss samples (that is, between sample collection and placement in the moss bag): (i) storing moss tissues in paper bags, air-dried at room temperature (20–25 °C); (ii) frozen storage (−18 to −30 °C). For persistent organic pollutants (POPs), it is recommended that the mosses be stored at 4–5 °C, in the dark, in polyethylene and/or polypropylene bottles/bags and stabilized as soon as possible. Among the literature we reviewed herein, however, there was no explicit reasoning for the choice of storage temperature. Only about 9.3% of the literature reviewed herein mentioned the exact specifics of the moss storage procedures, and only about 15.1% of these complied with recommendations.

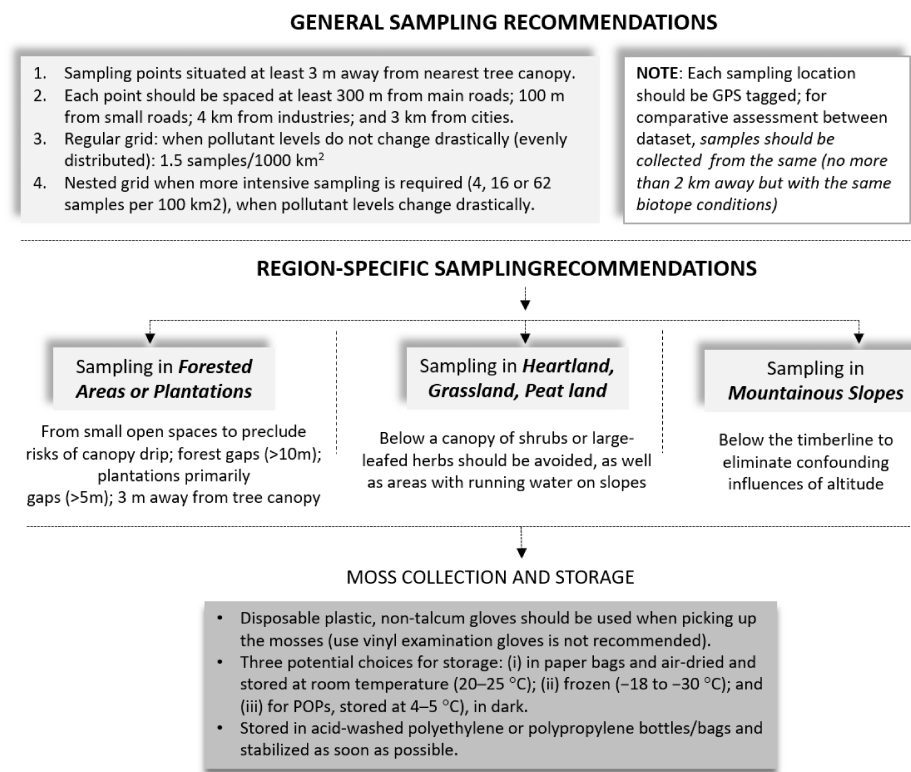


Figure 3. Recommended best practices for moss sampling (authors' own illustration).

3.3.2. Moss Part Selection

Regarding the specific parts of the moss to use for moss bag preparation, only about 53% of the literature reviewed herein clearly mentioned and recommended the exclusion of the brown parts of the moss, even if the green parts only represent the last two to three years of the moss tissue growth stages [101].

3.3.3. Moss Washing–Cleaning

Theoretically, a critical quality control step in the moss bag approach is washing and cleaning the moss (discarding foreign elements) before placing it in the bag [102]. About 91% of the literature reviewed in this study pointed to the importance of this, with about 43% of that literature stating that the moss samples were washed and cleaned as soon as they were collected to (i) avoid undesirable alterations in moss physiology, and (ii) avert undesired risks of microbial attacks and/or dust deposition on the moss surfaces.

However, despite acknowledging the importance of the washing–cleaning step (91% of the literature, as mentioned above) as an integral part of moss bag preparation, only a small fraction of that literature actually included all (2.09%) or some (15.34%) of the procedural details; over 81% of the reviewed literature only vaguely mentioned that “after collection, moss tissues were thoroughly cleaned” (Figure 4a).

While water was the dominant moss washing–cleaning medium (~87% of the literature that mentioned ‘some’ details in Figure 4a), a fraction of that same pool of studies also performed acid washes (~9.2%) (Figure 4b).

For moss bag results to be replicable (and comparable across studies), moss washing–cleaning procedures should ideally be standardized with regard to at least the following points [29]:

- Number of washes (washing steps);
- Wash duration (total and at each step);
- Volume of water (total and at each step);

- Water type (e.g., deionized/distilled);
- Acid (type, strength, volume, number of steps);
- Shaking (applied or not; mechanical/manual; intensity).

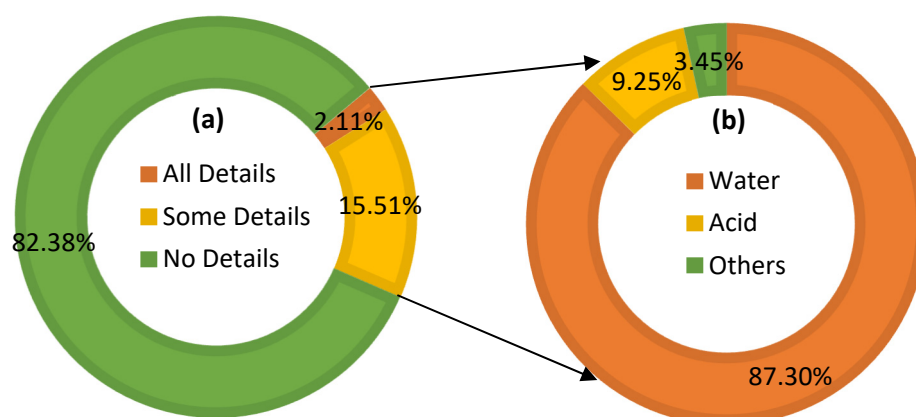


Figure 4. Percentage-wise distribution of moss bag literature reviewed herein that detailed the (a) moss washing–cleaning procedures and (b) leading from parts of panel ‘a’ (all + some details), various washing solvents used (authors’ own illustration).

In the literature mentioning acid washes, HNO_3 and HCl were dominant. Regarding the overall washing–cleaning step, Spagnuolo et al., the authors of [103], maintained that caution must be exercised because each method has its own concerns (Table 2). In a study specifically dedicated to optimizing the moss washing–cleaning method, Świsłowski et al. [104] tested four systems comprising the following:

- METHOD I: Removal of adhered materials/impurities (e.g., leaves, needles, soil).
- METHOD II: As above + conditioning mosses in demineralized water (each of 12 samples with a mass of 0.6 g d.m. was conditioned and left in 1 L of demineralized water with conductivity of $\kappa = 0.5 \mu\text{S}/\text{cm}$ for 1 h).
- METHOD III: As in Method I + averaging (manually mixing up the 12 moss samples taken from one measuring point and then separating them again).
- METHOD IV: As above + conditioning in 12 L of demineralized water with conductivity of $\kappa = 0.5 \mu\text{S}/\text{cm}$ for 1 h.

Table 2. Methodological constraints associated with various moss cleaning/washing protocols (authors’ own compilation from various reports).

Procedure	Methodological Concerns
Nitrogen Jet	• Irreversible loss of tissues, accompanied by physiological alterations • Potential loss of phyllids and/or rupturing • May alter the permeability of the plasma membrane • Less efficiency in removing extracellular particles
Power Ultrasonic	Potential loss of extracellular metals that are transferred to the water in which the moss is cleaned (overestimation of metal concentrations)
Chelating Agent (e.g., EDTA)	Mostly used as an extractant in the sequential elution technique to determine the elements present in the extracellular location

The Spagnuolo study [103] found Method IV to be the most appropriate for pre-exposure treatment. This is because mixing different samples of moss (mixing subsamples collected in the sampling site) produces more averaged material.

On the same note, only about 7.05% of the literature reviewed herein mentioned cautionary procedures while washing–cleaning, regarding the importance of maintaining the desired (low) initial levels of trace elements in the mosses prior to placing them in the bags [29]. In this regard, analyzing the washing solutions could considerably help to estimate the original concentrations of the analytes in the moss [34].

However, only about 11% of the literature reviewed in the present study explicitly discussed the process-level significance of moss cleaning:

- Clean the moss of edaphic particles and/or plant remains, so that the initial concentration of elements is as homogeneous as possible [22,74,76,105,106];
- Partially activate the tissues by removing some elements bound to cation exchange sites on the cell wall and membrane [107].

3.3.4. Moss Drying

Following the washing–cleaning and before placing the moss in the bags, the next important step is drying, which is necessary to remove excess moisture and/or other solvents. In the reviewed articles, the drying temperature varied widely; around ~15.8% of the literature stated 40 °C as optimal, at which the moss remains alive, while ~80.7% of the literature recommended 105 °C (constant dry weight) for better results [100,108,109]. However, there was no targeted discussion on the use of specific temperature(s). About 7% of the reviewed literature suggested that, for trace element monitoring, ‘oven-drying’ moss performs ‘better’ [96]. However, there was no reflection on the term “better” or any clear mention of the exact oven-drying temperatures used for the studies. For POP species analysis, freeze-drying is recommended [98]. About 4.2% of the reviewed literature recommended ‘acclimatizing’ the moss after drying for satisfactory results [107], especially for heavy metal analysis. For the latter case, dry-ashing was recommended, without the exact procedure for it being detailed [98].

3.4. Moss Bag Construction

The moss bag construction varied widely. For the bag material, preferences for nylon mesh (~70% of the literature reviewed), followed by polythene and mosquito net (Figure 5a), were observed. For the bag shapes, nearly 63% of the studies used cylindrical types (Figure 5b), while for the mesh net openings, the aperture dimensions varied from <1 mm² to 1–10 mm² (~80%, the most dominant aperture size) to over 10 mm² (Figure 5c). About 58% of the reviewed literature used bag sizes < 40 cm², while about 24% did not clearly mention the same, apart from stating “a suitable bag was constructed to hold the moss tissues” (Figure 5d).

The moss bag parameter choices (as listed above) could significantly affect the final bag output (e.g., ambient pollutant concentrations) (Table 3), indicating that universally accepted standard operating protocols (SOPs) are called for to avoid confusion (to improve replicability and reliability). Interestingly, only about 11.3% of the literature reviewed explicitly discussed the importance of these parameters, with most studies either not mentioning them at all or only briefly mentioning a few. For the latter, the choices of values varied widely.

Only a small fraction of the literature reviewed (~29%) clearly mentioned the ideal amount of moss to be placed in the bag; among these, about 48% suggested 3–5 g as the ideal amount, while about 30% vaguely mentioned an ‘optimal’ amount without specifying the exact value (Figure 6a).

Only about 26% of the literature reviewed mentioned details of moss packing, about 83% of which opted for ‘loose packing’; the rest, despite mentioning it as an important facet of moss bags, did not provide any specifications (Figure 6b). However, it should be

noted that both the moss amount and packing within the bag are critical for moss–pollutant interaction pathways for multiple reasons and should therefore be standardized to obtain replicable and reliable results (Table 3).

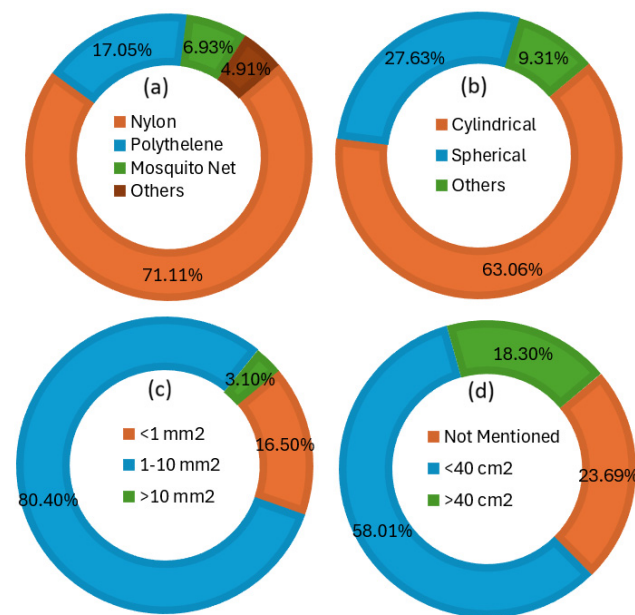


Figure 5. Percentage-wise distribution of the moss bag literature reviewed in this study for (a) bag material, (b) bag shape, (c) mesh net aperture dimension, and (d) moss bag size (authors' own illustration).

Table 3. Physical significance of various moss bag constructional parameters (authors' own compilation from various literature reports).

Aspects	Key Observations
Moss Bag Material	- Serves as the interface between the moss and ambient atmosphere, thus influencing pollutant interception–retention processes - Certain literature emphasized that non-plastic materials should be avoided, as they may interfere with the pollutant uptake process - Main variants include (i) nylon, (ii) polythene, (iii) polypropylene, and (iv) glass fiber; other types include mosquito nets, cotton, metal, and non-plastics
Moss Bag Mesh Net Opening Size	- Mesh opening size should be set to optimally balance pollutant interception capacity against potential leaching losses (rains) and/or losses due to wind/storm events - Varying from <1 to >150 mm² ~13% of literature reviewed herein included discussions on the physical significance
Moss Bag Shape	- Three main variants: (i) cylindrical (3-D), (ii) spherical (3-D), (iii) others (flat/square/rectangular; 2-D) 21.6% of the reviewed literature discussed the process-level significance of bag shapes: - A 3-D shape has mechanistic advantages, allowing uniform pollutant capture from all directions - In 2-D bags (flat), the moss's exposure to the atmosphere is more uniform 19.04% of the literature recommended the 'compartmentalization' of moss bags, which (i) prevents displacement of moss inside the bag, and (ii) keeps moss from getting flattened on the bottom (which would reduce pollutant capture efficiency)
Moss Bag Size	- Signifies the ratio between the moss weight and bag surface area (critical for pollutant capture efficiency) - A low ratio of moss weight to moss bag surface area is recommended for increased pollutant interception - Ratio varied from <40 to ~110 mg.cm^{−2}

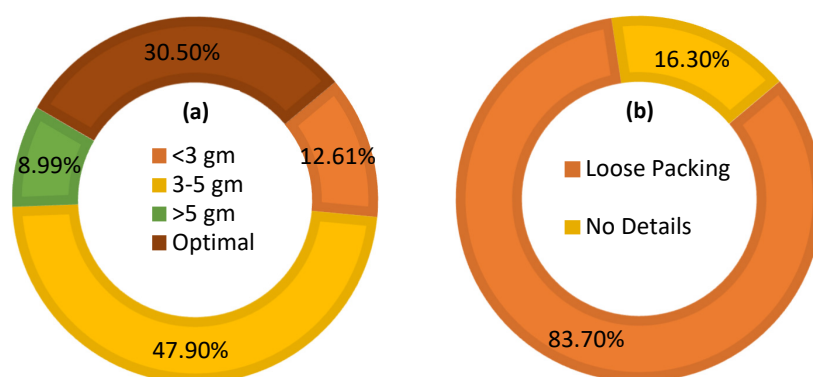


Figure 6. Percentage-wise distribution of the moss bag literature reviewed herein for (a) moss amount and (b) packing within the bag (authors' own illustration).

The most preferred bag exposure duration, among the literature reviewed in this study, was 4–6 weeks (~59% of the literature reviewed in this study) (Figure 7a), while for long-term pollution detection studies, exposure periods of over 8 weeks are also not uncommon (accounting for about 30% of our reviewed literature) (Table 3).

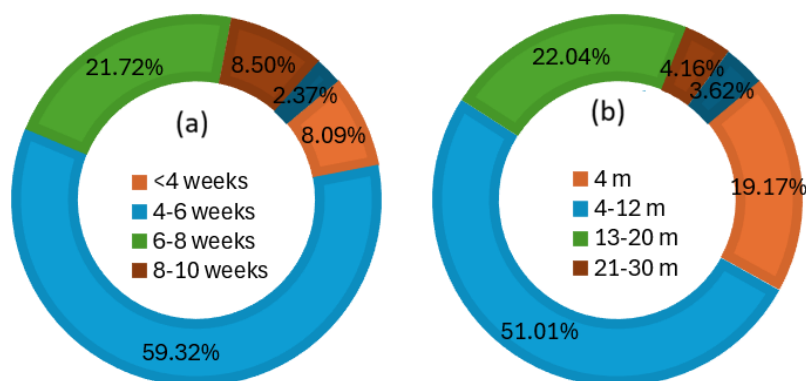


Figure 7. Percentage-wise distribution of the moss bag literature reviewed herein for (a) bag exposure duration and (b) bag elevation (authors' own illustration).

The moss bag elevations ranged from <4 m to >30 m above ground level, while the most preferred choice was between 4 and 12 m (~52% of the literature reviewed), followed by 13–20 m (24% of the literature) (Figure 7b). About 17% of the literature reviewed herein reflected on the importance of moss bag elevations: for example, while shorter elevations are more common for urban studies (e.g., traffic-related vehicular emissions), higher elevations are used more for assessing long-ranged pollution dispersion processes (Table 4).

Table 4. Critical considerations for setting up moss bags (authors' own compilation from various literature reports, as discussed in the main text).

Bag Details	Key Observations
Moss Amount	- Critical for moss packing within the bag and pollutant interception/uptake efficiency - Best recommended practice is to ensure adequate moss to brace against potential moss tissue loss during exposure as well as sample preparation for chemical analysis

Table 4. Cont.

Bag Details	Key Observations
Moss Packing	- Signifies the moss density (and porosity) within the bag - Critical for physico-chemical reaction mechanisms such as capture and retention (diffusion and conduction) of pollutants - Also influences pollutant leaching as well as moss tissue loss ~87% of literature advocates loose packing, comprising thin layers to maintain optimal porosity (reduced leaching losses) - Necessary to avoid overlapping and compression of moss layers to ensure optimal moss exposure for pollutant interception–uptake - None of our literature reviewed herein mentioned specific details of optimal moss thickness and/or number of layers
Moss Exposure Duration	- It might take the moss sample considerable time to reach a state of equilibrium at the study site - Should be determined by the project objective (long-/short-term monitoring) - Also, should be based on (a) emission rate and types, and (b) pollutants of interest
Moss Bag Elevation	- Highly variable, mostly guided by study objectives ~18% of the literature reflected on the physical significance: - Air circulation and turbulence vary enormously with elevation above the ground, in turn affecting pollutant dynamics - Should be guided by research objective: lower elevations (e.g., <4 m) aim to identify pollutant sources close to ground (e.g., vehicular emissions, storm dust), while higher elevations are preferred for long-range pollutant transport studies

4. Discussion

4.1. Identifying Ideal Moss Species

The literature revealed that moss species are selected based mostly on three broad considerations [29]:

1. Moss surficial properties;
2. Moss structural traits;
3. Pollutant type.

Moss Surficial Properties: Among the literature reviewed in this study, the significance of the moss surficial structures/compositions was the most cited concern, arising in about ~69% of the documents, pointing to its importance in ideal moss species selection. To that end, about 95% of the literature (out of the 69% from the above) pointed to the importance of the cation exchange capacity (CEC) of the moss cell wall/membrane as the governing factor for deciding on an ideal moss species. The CEC is a collective expression of all the different types of functional groups occurring on moss cells, including phosphodiester, carboxyl, phosphoryl, uronic and polygalacturonic acids, and polyphenols [29,95,110–113]. While most of the moss literature (about 82%) pointed to the carboxyl and phosphoryl species (rendering higher CEC values to the moss surfaces, thus increasing the pollutant/metal-binding capacity) [112,114], the importance of other functional groups, such as sulfhydryl and amine, is also mentioned [114].

Moss Structural Characteristics: About 38% of the literature reviewed mentioned the importance of the structural attributes of the moss for species selection, about 41% of which were more inclined to the epiphytic over the phylloid moss varieties. For example, the literature shows that the *Orthotrichaceae* sp., whose trunk is epiphytic, is more sensitive to atmospheric pollution than the *Polytrichaceae* and *Hylocomiaceae* varieties [115]. This is

due to the moss morphology, leaf area, leaf number, and stomatal abundance, and their aperture openings [116].

Pollutant types: In addition to the moss characteristics, another critical consideration for ideal species selection is the target pollutants—the form of pollutants (dissolved and/or particulate forms) and composition (valence and sizes) in the ambient atmosphere [62,117,118]. Based on the size and chemical nature of the pollutants, certain studies have even attempted to establish a sequence (order) of metal uptake by mosses [101,119]: lead (Pb) > cobalt (Co), chromium (Cr) > copper (Cu), cadmium (Cd), molybdenum (Mo), nickel (Ni), vanadium (V) > zinc (Zn) > arsenic (As).

In this regard, moss–pollutant interaction pathways also depend on the ambient ecological conditions [120]. Given the complexities, there are even recommendations for the use of different species for different pollutants [19,75,121]. However, a prime issue to resolve therein is the inter- and intra-species variation in pollutant uptake capacity, or differences between species grown in different habitats or in the same area but collected at different times [122].

4.2. Moss Bag—Infrequently Reported Parameters

Only a small body of the reviewed literature (13.18%) mentioned certain aspects of moss bags that could affect the outcomes. The following sections briefly reflect on some of these parameters.

4.2.1. Moss Bag Irrigation

The main argument behind irrigation practices is that, without the supply of ample water, moss specimens within the bag may dry up faster and eventually die and/or lose their capacity for pollutant interception [109,123]. Current research identifies this to be a risk factor, particularly for the *Sphagnum* sp., which is prone to drying out without a regular supply of water [51,76,104,105]. While Ares et al. [29,30] maintained that strategic irrigation helps to offset hydric and solar radiation stresses, Aničić et al. [95] maintained that the permanent irrigation of moss enables the binding of heavy metal ions at the ion exchange sites of moss surfaces.

Regarding the latter, in a study in Gdańsk City, Poland, using *Sphagnum palustre* moss bags, Astel et al. [124] noted that, in un-irrigated conditions, dry moss had a very loose collection of atmospheric particles, which led to moss tissue losses to rain/storm events. In the case of irrigated moss, on the other hand, pollutants become more incorporated within the moss tissues, thus withstanding any wash-off risks. For example, in a study in urban Belgrade, Serbia, Popovic et al. [125] observed that, although both dry and wet moss bags (*Sphagnum girgensohnii*) were capable of intercepting various trace metals, the irrigated moss had a higher metal interception–accumulation capacity (Pb, Al, Fe, Zn). The study [125] found that the Cr and Cu contents in the wet types were about eight- and two-fold higher, respectively, than in the dry one.

In this regard, a critical consideration is the mode of irrigation: auto vs. periodic (Table 5). For example, Mariet et al. [126] demonstrated that intermittent/periodic spraying (e.g., twice weekly) maintains sufficient moss hydration for bioaccumulation without the structural complexity of continuous auto-irrigation devices, establishing that the irrigation frequency must align with local atmospheric exposure conditions.

Automated irrigation systems utilize capillary wicks or water reservoirs to maintain a constant hydration state, which preserves active cell wall exchange sites and prevents cellular membrane collapse during hot or dry exposure campaigns [29]. However, studies also found that constant saturated states might raise the risk of elemental leaching, thus facili-

tating the wash-off of weakly bound particulate matter, whereas periodic irrigation allows for natural dry-deposition entrapment while avoiding excessive water throughput [127].

Table 5. Critical considerations for setting up various irrigation systems for moss bags (authors' own compilation from various literature reports).

Parameter/Feature	Auto-Irrigation (Continuous Wick/Reservoir)	Periodic Irrigation (Intermittent Spraying)
Hydration Mechanism	Constant capillary water supply from an attached reservoir	Scheduled manual or automated spraying (e.g., 1–2 times/week)
Cation Exchange Capacity (CEC)	Preserves steady-state ion exchange by preventing membrane collapse	Fluctuates based on wetting/drying cycles and cell permeability
Particulate Matter ($PM_{10}/PM_{2.5}$) Interception	Higher initial capture velocity due to continuously wet leaf surfaces	High passive physical trapping during dry intervals; re-suspended easily
Element Leaching/Wash-Off Risk	High risk of leaching weakly bound elements during prolonged saturation	Low risk of water-induced element leaching
Desiccation Risk	Extremely low (ideal for high-temperature/low-humidity sites)	Moderate to high if dry intervals are too long
Biological Integrity (Live Moss)	Maintains photosynthetic vitality and metabolic active uptake	Moss may enter physiological dormancy between wetting events
Optimal Study Context	Arid, semi-arid, hot urban street canyons, or multi-month dry summer campaigns	Humid environments, short exposure windows (1–4 weeks), or remote field sites

In effect, deciding on the moss bag irrigation mode calls for more process-level research. No single irrigation protocol is globally optimal; rather, auto-irrigated bags excel in dry, highly polluted street canyons, while periodic or passive exposure yields cleaner background signals in humid conditions [39]. Overall, two fundamental considerations in moss bag irrigation include (i) the time span and (ii) the season of the study. For example, for long-term studies, periodic irrigation could be necessary to keep the moss tissues alive and in a viable state to intercept pollutants. Similarly, during summer months, there would be a greater need for irrigation. The main caution to be exercised is not to 'over-irrigate' the moss tissues, creating artificial hydrologic stresses.

4.2.2. Moss Bag Orientation

Wind velocity and direction play critical roles in governing the patterns of the dispersion of air pollutants in the ambient atmosphere [35]. This relates to the pollutant uptake capacity with respect to the prevalent wind direction that blows the pollutants [40,77]. However, the bag orientation factor becomes critical with the choice of the bag shape [121]. For example, in flat bags, if pollutant flow occurs from a specific direction, moss uptake is largely governed by the orientation of the bag with respect to the prevailing wind direction. For flat bags, two possibilities arise:

- Vertical orientation: It intercepts pollutants from the horizontal wind flow (important when the idea is to monitor pollutants from a specific, known source).
- Horizontal orientation: It increases the likelihood of pollutant interception by gravitational sedimentation and wet deposition (this will require the arrangement of some support system).

For spherical/sub-spherical/cylindrical bags, on the other hand, pollutant interception occurs from all directions regardless of orientation. Only about 6% of our reviewed literature explicitly commented on the orientation details. A major reason could also be that most of the studies used spherical/cylindrical bags.

4.2.3. Moss Bag Shading

Optimal shading arrangements significantly affect moss bag biomonitoring outcomes [29,31]. At the process level, shading offers physical protection to the bags while

mitigating extreme environmental stressors such as direct solar radiation, heavy rainfall scour, and wind desiccation. However, by the same token, protective shading can obstruct coarse particulate matter from impacting moss surfaces, thereby altering the natural accumulation/moss interception kinetics [39,118]. Furthermore, shading can introduce variability into pollutant accumulation by creating artificial micro-spatial heterogeneity across the moss sample, such as localized water dripping points and an altered evaporation rate [40]. Despite these potential methodological artifacts, only a negligible fraction (~2%) of the active biomonitoring literature explicitly details or standardizes shading arrangements during field exposure [29].

4.2.4. Potential Influences of Meteorological Parameters

Ambient meteorological conditions alter the chemical uptake and physical trapping mechanisms of mosses (Table 6). About 13.6% of the literature reviewed herein explicitly mentioned the importance of meteorological factors for moss bag results [128]. To obtain a ‘representative’ estimate of air quality, there is a need to be mindful of the local meteorological conditions (e.g., air temperature, rainfall, wind speed and direction, barometric pressure, solar radiation, and humidity (absolute and/or relative) should duly be accounted for) [121,129]. For example, designing moss bag experiments by considering the local wind patterns (e.g., velocity and direction) is deemed especially critical for urban microenvironments due to the occurrence of artificially built-up, ‘enclosed’ spaces (e.g., parks, school/factory compounds, road tunnels) where natural air circulation is limited and unpredictable [16]. As a result, the pollutant concentration levels in these environs could be highly variable, both spatially and temporally.

Table 6. Broad overview of influences of various meteorological factors on moss–pollutant interception processes (authors’ own compilation from various studies).

Meteorological Factor	Moss–Pollutant Interaction and Physical Mechanism	Moss–Pollutant Interactions
Precipitation	- Pollutant Wash-Off: Surface particulate matter (PM_{10}, $PM_{2.5}$) is mechanically rinsed off moss phyllids. - Pollutant Leaching: Water-soluble ionic forms of heavy metals (e.g., Cd, Cu, Zn) undergo desorption and leaching.	Causes severe underestimation of dry deposition; creates high temporal variability during rainy exposure periods.
Wind Speed	- Boundary Layer Shrinkage: High wind speeds compress the boundary air layer, increasing the particulate inertial impaction velocity. - Blow-off: Extreme wind gusts cause re-suspension and mechanical loss of coarse particulates.	Modulates interception efficiency; higher wind speeds yield higher particle flux capture, but extreme winds reduce coarse particle retention.
Relative Humidity	- Hydration & Swelling: High humidity expands leaf surface area and keeps cell wall polygalacturonic acids active. - Cation Exchange Capacity (CEC): Active CEC binds dissolved metallic cations efficiently.	Enhances chemical bioaccumulation of dissolved trace elements; dry conditions force reliance solely on passive physical particle trapping.
Air Temperature and Solar Radiation	- Desiccation: Heat causes rapid loss of cellular turgor and membrane damage in living mosses. - Photo-oxidation: High UV/solar radiation degrades surface-adsorbed polycyclic aromatic hydrocarbons (PAHs).	Causes underestimation of organic pollutants (e.g., PAHs) due to photo-degradation and thermal volatilization.

To account for/minimize potential meteorological bias, standard active biomonitoring protocols often recommend ‘normalizing’ the elemental concentrations using rain gauges/meteorological station data, employing devitalized mosses to avoid physiological variability, and keeping exposure durations between 6 and 12 weeks.

4.3. Anticipated Future Research and Development

Air quality data obtained from moss bags could help in meeting multiple UN Sustainable Development Goals (SDGs) (Table 7). However, given the wide-ranging variability, for the moss bag results to influence air quality decision-making, there is still a need

for more targeted studies [30,31,39,40,61] to ‘standardize’ the parameterization process with pilot-scale ‘sensitivity experiments’ identifying the best combinations of parameter values [46,49,105]. In addition, there is the need to compare moss bag results with those of traditional, instrument-based air quality studies [95,130,131] and characterize the differences and uncertainties therein.

Table 7. Targets under various UN Sustainable Development Goals, potentially linked to poor air quality and related public health hazards, where moss bags could help (authors’ own conceptualization).

UN SDG	Selected Target Areas
3 (Ensuring Good Health and Well-Being)	3.1—By 2030, reduce the global maternal mortality ratio to less than 70 per 100,000 live births 3.9—By 2030, substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water and soil pollution 3.D—Strengthening national capacity, especially in developing countries, for early warning, risk reduction, and management of health risks
7 (Clean Energy)	7.2—By 2030, increase substantially the share of renewable energy in the global energy mix 7.A—By 2030, enhance international cooperation to facilitate access to clean energy research and technology, including renewable energy 7.B—By 2030, expand infrastructure and upgrade technology for supplying modern and sustainable energy services for all in developing countries, in particular least developed countries, small island developing States
11 (Sustainable Cities and Communities)	11.6—By 2030, reduce the adverse per capita environmental impact of cities, including by paying special attention to air quality and municipal and other waste management 11.7—By 2030, provide universal access to safe, inclusive and accessible, green and public spaces 11.B—By 2020, substantially increase the number of cities and human settlements adopting and implementing integrated policies and plans towards inclusion, resource efficiency, mitigation and adaptation to climate change
13 (Climate Change Action Planning)	13.2—Integrate climate change measures into national policies, strategies and planning 13.3—Improve education, awareness-raising and human and institutional capacity on climate change mitigation, adaptation, impact reduction and early warning 13.B—Promote mechanisms for raising capacity for effective climate change-related planning and management

4.3.1. Towards a Standard Operating Protocol (SOP)

Given the range of variation in moss bag preparation procedures in the literature, there must be targeted efforts to establish a standardized operating protocol (SOP) for moss handling, bag construction, and bag exposure routines [27,50,53,77,130]. Although such efforts are currently underway, they are scanty at best. For example, in a study, Capozzi et al. [39] attempted to build a ‘concept’ moss bag, namely the “Mossphere” (as part of the FP7 European MOSSclone project), using various values for different parameters to test their effects on moss bag performance:

- Moss Species: *Pseudoscleropodium purum* (Hedw.) M. Fleisch.
- Bag shape (three main variants in the literature reviewed herein—round, spherical, and flat).
- Mesh material (nylon).
- Mesh opening size (three main values: 1, 2, and 4 mm).
- Moss weight/bag surface area ratio (three main values: 15, 30, and 45 mg cm^{−2}).
- Bag exposure period (three values: 3, 6, and 12 weeks).
- Bag elevation (three main values: 4, 7, and 10 m above ground).

While conducting the study across three climatic regions in Europe (oceanic, Mediterranean, continental) and three ecosystems (urban, industrial, agriculture), the authors [39] found the following to be the most influential on the results:

- Density within moss bag: the lower the moss density within the bag, the higher the pollutant uptake potential.

- Moss weight to bag surface area ratio: between 5 and 15 mg/cm², which allows a more homogenized distribution of elements on the moss surface, thus minimizing risks of analytical variations.
- Bag exposure period: between 6 and 12 weeks, to allow homogenization of pollutants.
- Moss bag shading.

In a similar effort to optimize the moss bag methodology, Ares et al. [31] recommended paying special attention to four main aspects:

- Moss species:
- Moss Weight–Surface Area Ratio: This denotes the functional area exposed to air.
- The rougher the surfaces, and if coupled with hairiness (phyllid), the higher the pollutant uptake capacity and the lower the risks of pollutant losses, e.g., by blow-off or bounce-off and/or rain wash.
- A higher number of phyllids per caulid also increases pollutant uptake [132,133].
- Exposure Period: About 8 weeks preferably, although the literature shows that the exposure period varied from 30 to 45 days [29].
- Bag Elevation: Placement of moss bag about 4 m above ground level.
- Should be guided by the research objective; for example, for traffic-induced emissions, moss bags should be exposed closer to ground level, where they can assess human exposure [45].

The choice of exposure period is deemed a critical factor in moss bag outputs. Although most of the literature reviewed herein recommended short-term exposure (weeks to months), recent studies that use longer ones are emerging. For example, in a study of a suite of PAHs and trace elements, Rajfur et al. [123] and Swislawski et al. [134] exposed *P. schreberi*, *S. fallax*, and *D. polysetum* bags over a 12-month period. Both studies observed significant pollutant interception by all three moss species, which increased over time.

In another such standardization experiment, in Sardinia, Italy, De Agostini et al. [51] exposed *H. cupressiforme* Hedw. moss bags near an oil refinery over a 16-year period, noting relatively stable accumulation of 16 trace elements (other than variations caused by changes in ambient meteorological conditions, such as wind directions and humidity).

A main concern about such long-term approaches is potential phytotoxic expressions due to prolonged pollutant exposure, and pollutant buildup within the moss cells, which could lead to harmful phytotoxic effects. Another potential concern is the risk of physical damage, i.e., the wear and tear of the moss bags themselves over longer periods.

4.3.2. Ensuring Moss Functionality and Viability

Moss Phytotoxicity

The literature reveals that a key concern is ensuring the long-term vitality of the moss tissues within the bag, that is, maintaining the moss cells in a healthy and functional condition throughout the exposure period [135]. This is critical because proteomic assessments of heavy and trace metal accumulation (e.g., Cr, Cu, Ni, Zn, Cd, Pb) by *Hypnum cupressiforme* moss bags near an oil refinery in Sardinia, Italy, revealed drops in protein expression with increased metal uptake, which included a variety of biomolecules such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), proteins of the light-harvesting complexes of photosystem (PS) II [119]. Heavy metals such as Cd²⁺ and Pb²⁺ are known to hinder water uptake and reduce essential hydrolytic enzyme activity.

Moreover, increased metal accumulation within moss tissues interfered with protein synthesis pathways, in addition to affecting stress-related proteins. While such adverse changes within the moss could be strategically monitored as early signs of ambient air qual-

ity shifts (e.g., early warning signs of air pollution), there is also the need for more targeted studies to improve our current understanding of moss's phytotoxic expressions [132].

Furthermore, efforts should also be made to elucidate the internal mechanisms that resist negative shifts. For example, certain intracellular metals (e.g., Cu, Zn) are necessary micronutrients for healthy moss growth, while others could be toxic (e.g., Pb, Cd) [136,137]. Basile et al. [136] found that *Scorpiurum circinatum* (Brid.) Fleisch. & Loeske survived various metal toxicities (Cd, Cu, Pb, and Zn), possibly by immobilizing most toxic ions extracellularly, likely in the anionic binding sites (various organic functional groups) in the cell wall and plasma membrane, which are the main sites of metal detoxification.

Future research in this area might involve controlled experiments to expose moss bags to a wide array of pollutant species at various concentrations to monitor the moss's response patterns.

Moss Chlorophyll Status

Regarding the risks of phytotoxicity, a fundamental aspect is the critical monitoring of quantitative moss chlorophyll contents [18,65,66,133,138–141]. These photosynthetic pigment variations are tightly dictated by the toxicant profiles, baseline exposure concentrations, and cellular influx kinetics. Mechanistically, toxicological research confirms that divalent heavy metal cations directly compete with essential macronutrients for membrane transport proteins, causing internal homeostatic failure and structural membrane disruptions [138]. Once localized inside energy organelles, excess heavy metals disrupt pigment integrity by competitively displacing the vital central magnesium (Mg^{2+}) ion from the chlorophyll porphyrin ring structure [139], yielding photochemically inactive metal–chlorophyll complexes that completely halt light-harvesting kinetics.

Concurrently, intense xenobiotic accumulation initiates a cascade of cellular oxidative stress and reactive oxygen species (ROS) overproduction [138]. This uncontrolled oxidative surge triggers an apparent 'conversion' phenomenon—typically characterized by a sharp drop in the chlorophyll a to b ratio—which stems directly from the targeted degradation of light-harvesting complex II (LHC II) subunits and accelerated lipid peroxidation of thylakoid membranes [138]. For instance, experimental exposure of the ectohydric moss *Pleurozium schreberi* to complex multi-metal matrices containing Cu, Zn, Cd, Hg, and Pb drives severe, widespread declines in total chlorophyll content. This structural pigment breakdown represents a downstream consequence of cellular physiological strain [140], directly shaped by functional morphological traits that determine metal exposure, retention, and cell-wall exchange capacity across the bryophyte canopy.

Moss Metabolites

A major future research area should be gaining better insights into the impacts of various metabolite species within moss cells induced by pollutant interception. For example, research on the *Sphagnum* sp. revealed that various pollutant uptake mechanisms may produce a wide array of metabolites [141] that have critical roles in moss physiology [142]. Common moss metabolites include carbohydrates, carotenoids (primary metabolites), phenols, proline, flavonols, and tannins (secondary metabolites). In various ways, these species regulate moss growth behavior and photosynthesis efficiency, and maintain cell structural integrity and resistance to decomposition under various abiotic stresses [143]. By the same token, if the production routines of these metabolites are disrupted (e.g., by the uptake of toxic metals), the entire moss physiology may be hampered, making the moss bag unviable.

Pollutant Fractionation Mechanisms

For the future research and development of active air quality biomonitoring using moss bags, another critical area of investigation is metal fractionation/apportionment within the moss tissues. The literature indicates three prime modes by which metals can become part of the moss tissues [112,114,144]:

- Intercellular Fraction: Pollutant species (e.g., metals) that are either adsorbed or in dissolved forms deposit around moss cells.
- Extracellular Fraction: Species bound in exchangeable forms to either exchange or chelating sites (organic functional groups) on the moss cell wall and outer surface of the plasma membrane [111].
- Intracellular Fraction: Species transported inside the cells and held in soluble and/or insoluble form [145]. This fraction is guided more by the saturation kinetics within the moss cell [136].

In this regard, the driving question could be as follows: Which mode of fractionation operates/dominates under various environmental–climatological conditions [100,145]?

4.3.3. Moss Bag: Environmental Variables

Drawing from the above, a critical area of interest could be the impacts of various environmental conditions on moss functioning [146]. In other words, what affects moss's vitality for pollutant interception? This is important because, after collection, the bags into which mosses are placed could have completely different environmental conditions compared to their place of origin, which may affect their functioning (e.g., ability to intercept pollutants) [38,106]. For example, research has shown that environmental stress can lead to the generation of various reactive oxygen species (ROS) [133,147], which may damage the living tissue within mosses. By the same token, the pattern of the production of antioxidant enzymes (e.g., ascorbate peroxidase, catalase, peroxidases, superoxide dismutase) as internal protection from oxidative damage [148] will also be important in future research to understand the long-term viability of moss tissue and functioning.

In a study using various *Sphagnum* moss species, Sytiuk et al. [149] maintained, “the exact linkages between *Sphagnum* anatomical, morphological and biochemical traits (i.e., metabolites, pigments, antioxidant enzyme activities) are yet not very well known, and neither are their collective response action pathways to changes in environmental conditions”. In this study [149], assessing how the *Sphagnum* anatomical, morphological, and biochemical traits (such as metabolites, pigments, and antioxidant enzymes) link together, the authors found that the inherent moisture levels (water-holding capacity) are critical drivers of these traits, establishing four main trait clusters: growth, biomass, defense, and water-stress tolerance. The research found that “the species with the highest water holding capacity also exhibited the highest antioxidant capacity, as showed by the high flavonoid and enzymatic activities in their tissues”.

5. Concluding Remarks

The mounting cases of public health hazards related to degrading ambient air quality around the world call for focused research and development to bolster and diversify monitoring and assessment capacity and efficiency to support more data-driven interventions. In this regard, the efficient use of moss bags can offer air quality managers/decision-makers multiple advantages over traditional sensor-based approaches for monitoring pollutant concentrations and their origins, alongside studying their spatial distributions.

The study showed that there is yet much to be learned about moss bags for them to become a potent decision-making tool for concerned air quality authorities around the world. The prime reason is the wide variability in moss bag operational procedures

(moss handling, bag construction, and exposure routines), with ‘user-defined customization’ approaches often employed, rather than a universally accepted (or recommended) standardized operating protocol (SOP) of parameters, which could jeopardize the moss bag results. As for the dominant moss species type found in the literature, the preference has been for the *Sphagnum* sp., probably due to its confluence of physiological and surficial features over other moss species. Regarding the moss bag preparatory steps, the cleaning–washing medium used (although water has been preferred in most cases) showed a great deal of variability across the literature, with no clear mention of moss sampling and storage protocols, and a wide range of moss drying temperatures.

The variability became even more apparent upon scrutinizing the bags’ constructional details: the bag material (plastic or nylon), bag size, bag shape (cylindrical or spherical), moss packing within the bag (loose or dense), and mesh opening sizes (<1 to >10 mm²). On occasion, the specific details of all the above parameters were not even mentioned explicitly. Furthermore, there have been wide-ranging choices for the moss bag exposure period (e.g., exposure duration, elevation). In addition, for several parameters, there has been no explicit mention yet in most literature (e.g., moss bag irrigation, shading, orientation during exposure), which could influence the results and question the replicability of studies.

Overall, this study could help to guide future research and development in the related fields, aiding in air quality decision-making. However, there remains a need to conduct targeted research to gain improved insights into several process-level aspects, including the various moss–pollutant interaction pathways under different environmental–meteorological conditions, for example, moss phytotoxicity, the pollutant partitioning mechanisms within moss cells, and the long-term sustainability of moss bags due to adverse alterations in moss chlorophyll contents.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/environments13090477/s1>. Table S1: Detailed elements in the PRISMA 2000 procedure, as adopted in this scoping review.

Author Contributions: Conceptualization, S.C. and M.R.; Methodology, M.R. and S.C.; Software, S.C. and M.R.; Validation, M.R. and S.C.; Formal Analysis, S.C. and M.R.; Investigation, M.R. and S.C.; Data Curation, M.R. and S.C.; Resources, S.C. and M.R.; Funding Acquisition, none applicable; Writing—Original Draft, M.R. and S.C.; Writing—Review and Editing, S.C. and M.R.; Visualization, S.C. and M.R.; Supervision, S.C.; Project Administration, S.C. and M.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: This is a Systematic Literature Review article, so no primary data was generated.

Acknowledgments: The authors cordially thank the Jindal School of Liberal Arts and Humanities, O.P. Jindal Global University, and the research staff of the Centre for Environment, Sustainability and Human Development (CESH), O.P. Jindal Global University, for providing assistance in performing the research.

Conflicts of Interest: The authors declare no conflicts of interest.

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